

Standard Operating Procedure

Bruker MultiRAM FT-Raman Spectrometer

1. Introduction

1.1. Purpose

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

1.2. 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.

This procedure is applicable to undergraduate and graduate students enrolled in courses within the Department of Physical and Environmental Sciences. This document may also be used as a template for research users within the Department of Physical and Environmental Sciences.

1.3. Responsibility

User

1.4. Accountability

Principal Investigator/Course Instructor/Teaching Assistant

1.5. Emergency Contacts

- Emergency Fire/Police/Ambulance:911
- UofT Police:416-978-2222

2. Referenced Documents

- 2.1. Bruker MultiRAM User Manual, 2nd edition.
- 2.2. Bruker OPUS Spectroscopy Software, Version 6
- 2.3. Bruker OPUS OVP Spectroscopy Software, Version 6
- 2.4. Bruker OPUS Quick Reference Guide, Version 7

3. Chemicals & Supplies

- 3.1. Bruker multiRAM FT-RAMAN Spectrophotometer
- 3.2. Bruker Raman Sample Kit*
- 3.3. Isopropanol or Ethanol
- 3.4. Kimwipes
- 3.5. Course-provided or Course-produced samples ONLY
 - **Use of this instrument for purposes other than laid out by the course instructor will result in severe penalty and academic offense.**

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4. Personal Protective Equipment

- 4.1. Nitrile/Nylon Gloves
- 4.2. Laboratory Coat/Jacket
- 4.3. Safety Glasses

5. COVID-19 Related Safety Precaution

5.1. Do not enter if you have one of the following symptoms:

- cough
- fever
- difficulty breathing
- pneumonia in both lungs
- travelled outside the country in the last 14 days

5.2. Cleaning and Sanitizing Hands

5.3. General Laboratory Practice during COVID-19

- You MUST work >2m from others. The use of adjacent instruments less than 2m is suspended at this time. Please schedule your analysis appropriately.

6. Safety and Electronic Equipment Concern

6.1. Chemical Safety

- Read and become acquainted with the SDS of all the chemicals you will be using and/or exposed to during the lab period -including the alcohols for cleaning.
- Dispose of the chemical waste and chemicals-soaked paper in the designated containers.

6.2. Electronic Safety

- Please refer to the manufacturer's recommendations and warning label.

6.3. Before Commencing work

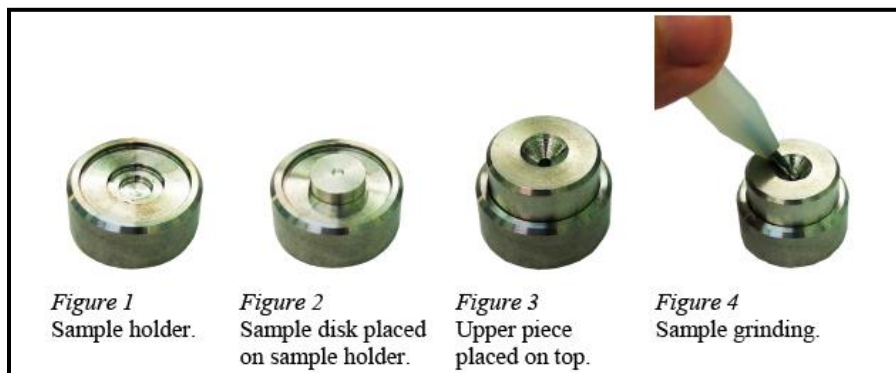
- Obtain a bottle of alcohol and several paper towels. Kimwipes should also be available near the Raman.
- Clean the keyboard, mouse, GLOVES, and any area you will be exposed to (monitor excluded) with the solvent-soaked paper towels. DO NOT spray directly onto surfaces.
- Discard ALL the paper towels (whether they were used or not) into the designated waste container.
- Wait 5 minutes before commencing work.

7. Raman Sample Preparation

7.1. Preparation of solid sample disk

- Prepare a fine-ground powder from the analyte using a mortar and pestle.
- Place a clean and dry sample disk on the sample holder (figure 2).
- Place the upper piece on top of the sample holder (figure 3).
- Add a spatula tip of the fine-ground sample and compress with the grinder (figure 4).
- 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).

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7.2 Preparation of liquid sample or powdered* solid

- Place the liquid or free-running powder into the 5mm quartz raman cuvette.
 - Fill to $\frac{3}{4}$ capacity
- Alternatively, you can use a standard 10mm quartz cuvette with a mirror end. *

7.3 Macro or Mineral Samples*

- Place macro or mineral rock sample inside the sample chamber.
- 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).
- Change position of the mineral till proper spectra can be acquired.

8. Acquiring Raman Spectra

8.1. Initialization of the instrument

- *N.B. Ensure the presence of sufficient liquid nitrogen(LN2) in the cooling dewar of the instrument. The LN2 in the dewar has a 3-5 day hold time and must always be replenished prior to initializing the instrument*.*
- The FT-Raman laser is controlled by a key-operated power switch with two turn-settings: standby and on.
- Upon the first turn, the 'Line' LED should be red, followed briefly by the 'Warm-up' LED (figure 6).
- Once the instrument is warmed up, the 'Warm-up' LED will turn off and the 'Standby' LED will turn amber (Figure 7).
- Proceed to turning the key to the second setting. The instrument is now ready for sample analysis (Figure 8).

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Figure 5 Off.



Figure 6 Warm-up phase.




Figure 7 Standby phase.



Figure 8 Instrument is ready.

8.2. Sample Analysis

- Proceed to run the OPUS software.
 - Connect to OPUS on the desktop
 - Username and Password are required
 - Ensure that communication between the software and instrument are established
 - Green Dot should appear at the bottom right corner of the OPUS software
- Lift open the sample chamber (figure 9).
- Secure the disk/cuvette on the stage making sure the sample surface is facing the lens (figure 10).
- With cuvettes, ensure the mirror is placed behind the cuvette facing the lens.
- Close the sample chamber.
- Click on 'Advanced Measurement' 
- In the dialogue box that appears, enter the sample description and adjust the Raman laser power (10-500mW), 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.*
- Proceed to the 'Measurement' tab and select 'Check Signal'. Proceed to click 'Spectrum' in the 'Show' settings. This gives a preliminary view of the Raman spectrum.
- Laser power can be adjusted on the scale to allow for maximum signal strength.

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- Use the 'Forward' and 'Backward' buttons to adjust the sample on the z-axis. This is used to maximize signal strength.
- Once done with these manipulations, click 'Sample Raman Spectrum' (Figure 11) and wait for the generation of the spectrum. The green bar indicates the progress of the measurement.



Figure 9 Open sample chamber.



Figure 10 Placement of sample disk on the stage.

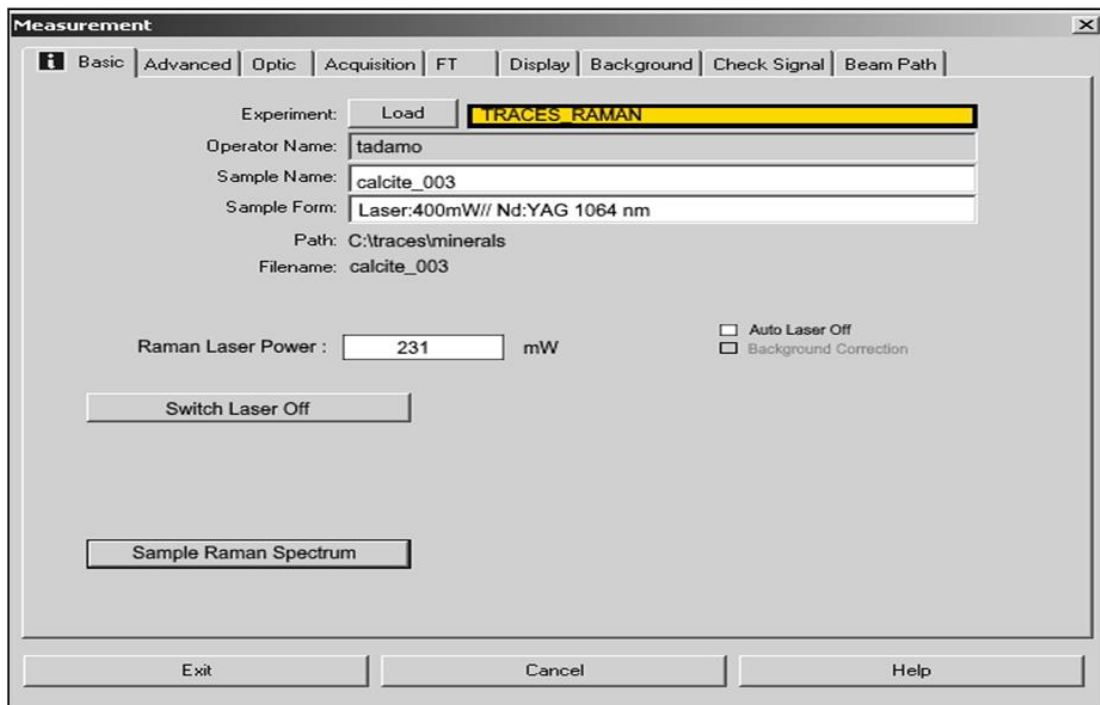






Figure 11

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9. Raman Data Evaluation*

- With the sample spectrum open in OPUS, click on 'Peak picking' . In the dialogue box that appears, click 'Start Interactive Mode'.
- Adjust the horizontal line to be just under the smallest peak of interest.
- 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).
- Alternatively, Right-click mouse and choose Single Peak Pick for manual selection.
- If the sample requires Baseline Correction click the icon .
- Click on the icon  to print your spectra.
- Click on the icon  to remove your spectra from the workspace.

10. Waste Disposal

- All waste generated during the lab are to be disposed of in the appropriate waste container.

***The TRACES Manager will provide further details during hands-on training.**