Standard Operating Procedure: Fourier-Transform Infrared Spectrometer (FT-IR)

Thermo Fisher Nicolet iS50 Spectrometer

December 2025

Contact Information:

Shawn Wagoner (swagone@gmu.edu)

Sezin Sayin (ssayin@gmu.edu)

Table of Contents

1. Lab Safety Information

- ✓ All GMU NFF users are required to complete the Lab Safety Orientation (LSO) before performing any lab work.
- ✓ Proper Personal Protective Equipment (PPE) should always be worn before entering the clean room: safety glasses, hair net, shoe covers, gloves, and lab coat. Additional PPE is available for specialized chemical work as needed.
- ✓ No shorts, sandals, tank tops, or spaghetti-strap shirts are allowed in the clean room!
- ✓ Material Safety Data Sheets (MSDS) are available in a binder in the gowning room.
- ✓ Read the SDS for any chemicals you plan to use before proceeding with your work. Any materials used in the clean room for the first time should be brought in after the approval of NFF staff.
- ✓ A safety buddy is required in the clean room with you when doing chemical work. The safety buddy should be fully trained and qualified to work with the chemical you are using. They must remain in the clean room the entire time you are handling the chemical. Feel free to ask NFF staff if no one qualified is available!
- ✓ Prohibited clean room items: cardboard, pencils, cloth, hats/coats, and contact lenses.
- ✓ Accepted clean room items: plastic, pens, synthetic fabrics, clean room paper.

2. FT-IR Safety Information

- ✓ Fill out the logbook before you begin.
- ✓ Any irregular system behavior should be reported to NFF staff promptly. Never attempt to fix the system yourself! We are here to help.
- ✓ The spectrometer should be handled carefully, do not force the ATR module knob too tightly.
- ✓ When measuring sample on the Diamond ATR module, ensure the sample is secure and tight.
- ✓ Ensure that the ATR module is clean after your measurement, both the diamond crystal plate and the pressure tower.
- ✓ The laser light within the sample compartment cover should not be stared at directly.
- ✓ Avoid exposure to potentially hazardous materials, prevent damage to the instrument.
- ✓ Avoid scratching or damaging the ATR crystal during sample preparation and ensure it is clean before use.
- ✓ Dispose your solvents and waste materials according to established protocols.
- ✓ Maintain a clean work area and ensure the instrument's optical components are free from dust and contaminants.
- ✓ Failure to use the system safely and properly may result in your access to the system being reviewed and/or revoked.

3. Principles of FT-IR

FT-IR Spectroscopy is an analytical technique used to identify organic, polymeric, and some inorganic materials by analyzing how molecular bonds absorb infrared (IR) light. The process uses a Michelson interferometer, which splits a beam of IR light using a beam splitter. These beams are reflected by a fixed and a moving mirror, then recombined to produce interference patterns. As the moving mirror shifts, the phase difference changes, producing an interferogram, a record of light intensity versus optical path difference.

FTIR detects unique absorption patterns or "fingerprints" of different molecular bonds, enabling to determine molecular structure and composition. The equipment shines IR frequencies onto a sample and measures how much each is absorbed.

At GMU's NFF, the FTIR system includes transmission, ATR, and NIR modules, supported by data libraries and processing software for efficient analysis and data storage.

ATR module: allows analysis of powder or liquids samples without sample preparation.

NIR module with integrating sphere: enables quick, consistent measurements of solids and liquids in containers or in situ, even for heterogeneous samples.

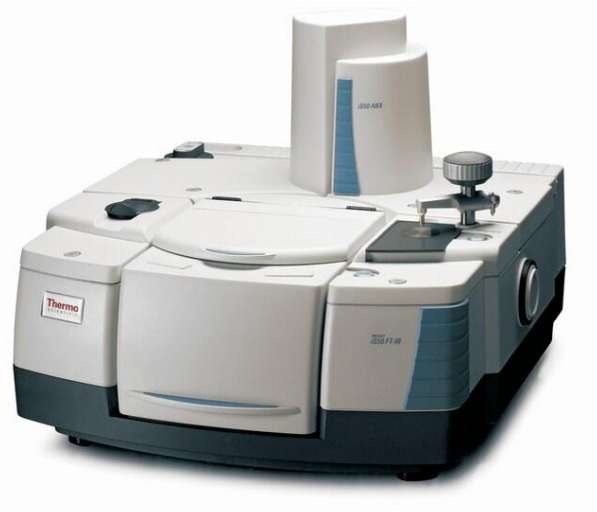

Figure 1. Nicolet™ iS50 FTIR Spectrometer

4. Attenuated Total Reflectance (ATR) Module Operation Manual

4.1. Stability Test

1. Clean diamond crystal plate and the pressure tower with the provided tissue paper and minimal amount of isopropanol.
2. Click the OMNIC icon to launch the software.

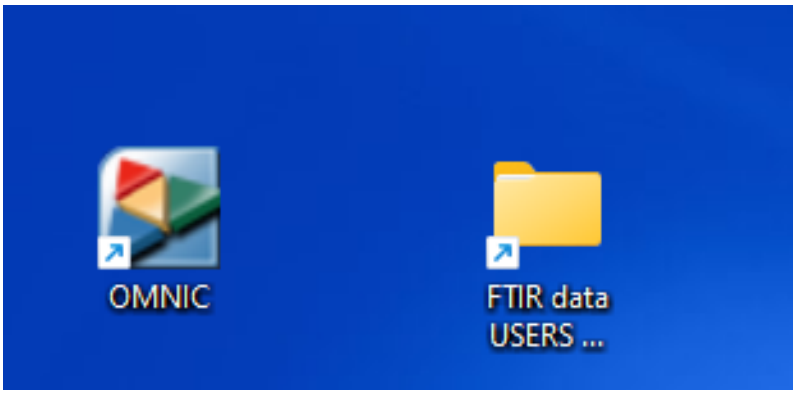

Figure 2. Omnic Windows Shortcut

3. You might encounter an error during the default “stability test”. In that case, you need to perform a laser alignment in transmission E.S.P. mode.

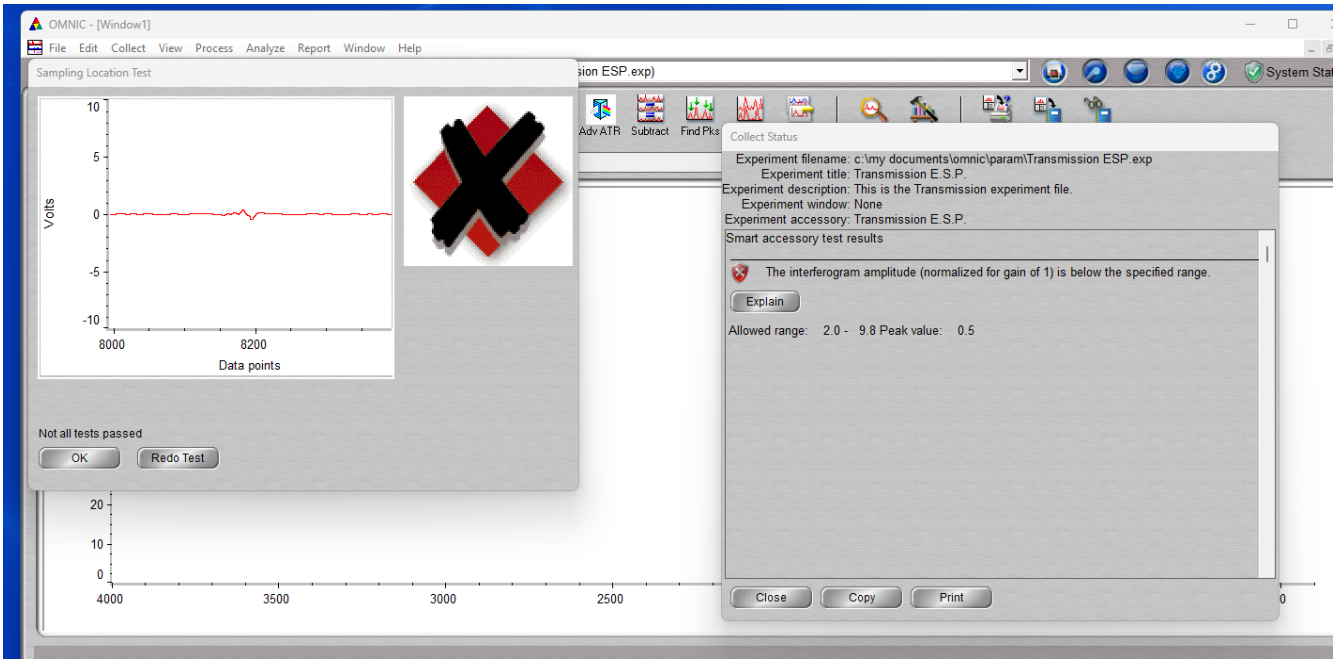

Figure 3. Initial Diagnostic Pop-Up

4. To perform laser alignment, close all the pop-up windows and then go to Collect > Experimental set-up> A pop-up window will appear> Diagnostic> click Align and wait for few minutes.

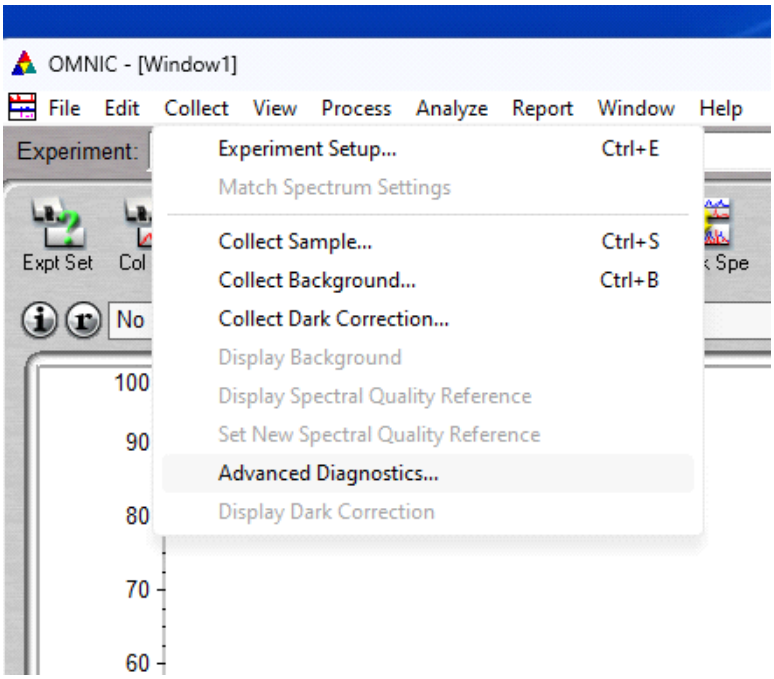

Figure 4. Collect Menu

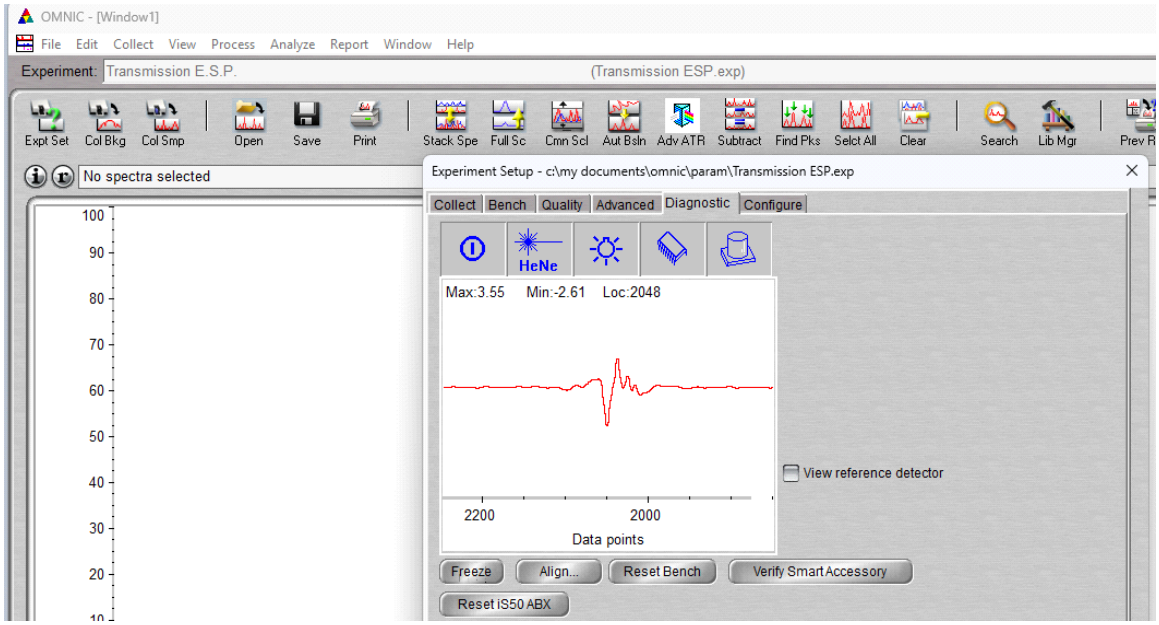

Figure 5. Experiment Setup Diagnostic Menu

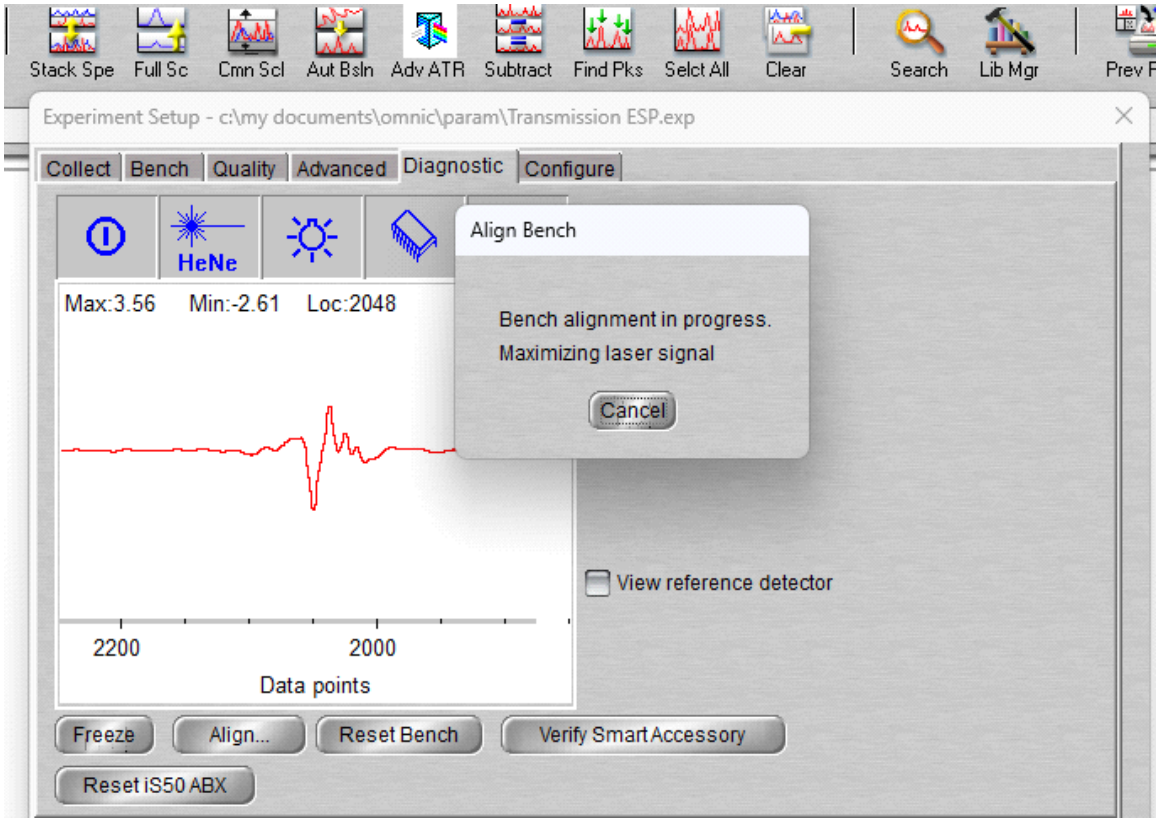

Figure 6. Laser alignment in progress dialog

5. After alignment is completed, click OK and close the pop-up tab, then close the software. After alignment, experiment can be started. This step is required only for stability test error.

4.2. Launching Software and Selecting Experiment

6. Start the OMNIC software and the alignment pop-up will be displayed (green check mark). These tests run automatically, no need to click anything in pop-up window. Once the test is successful all the pop-up windows disappear, leaving behind the main software panel.

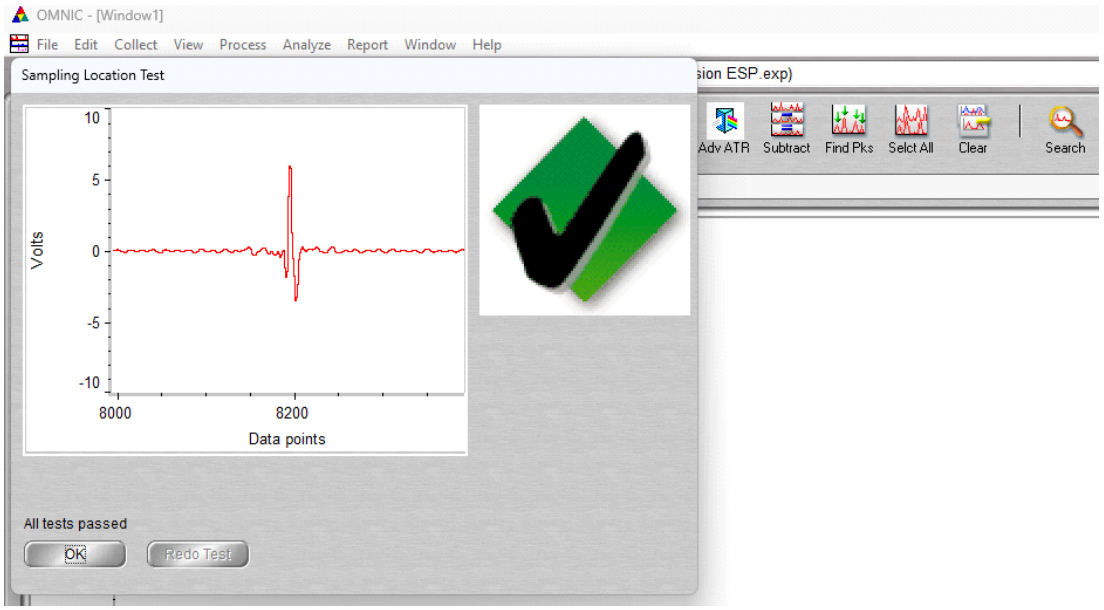

Figure 7. Sampling Location Test Menu

7. Select IS50ATR from the drop-down on the top to switch the instrument from Transmission (default) to ATR mode. You will see the blue button glowing now on the instrument near the "iS50 ATR" label. Wait until the blue light becomes stable, not blinking.

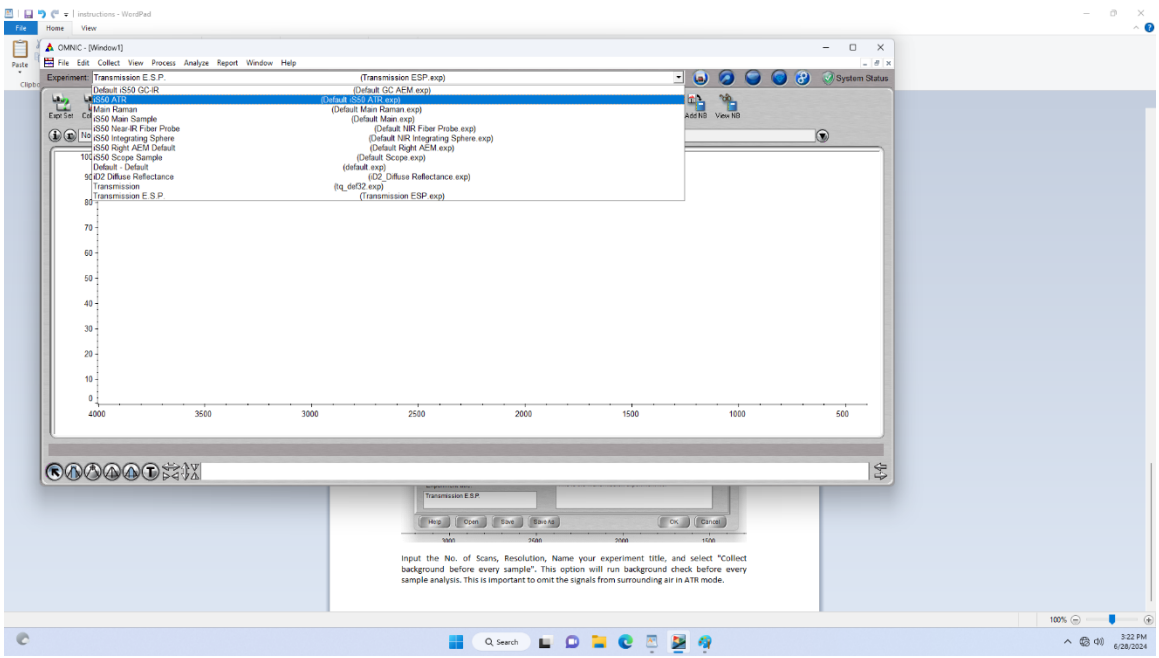

Figure 8. IS50ATR option in drop down menu

4.3. Experiment Setup

8. For measurements - Go to Collect > Experimental set-up> A pop-up window will appear.

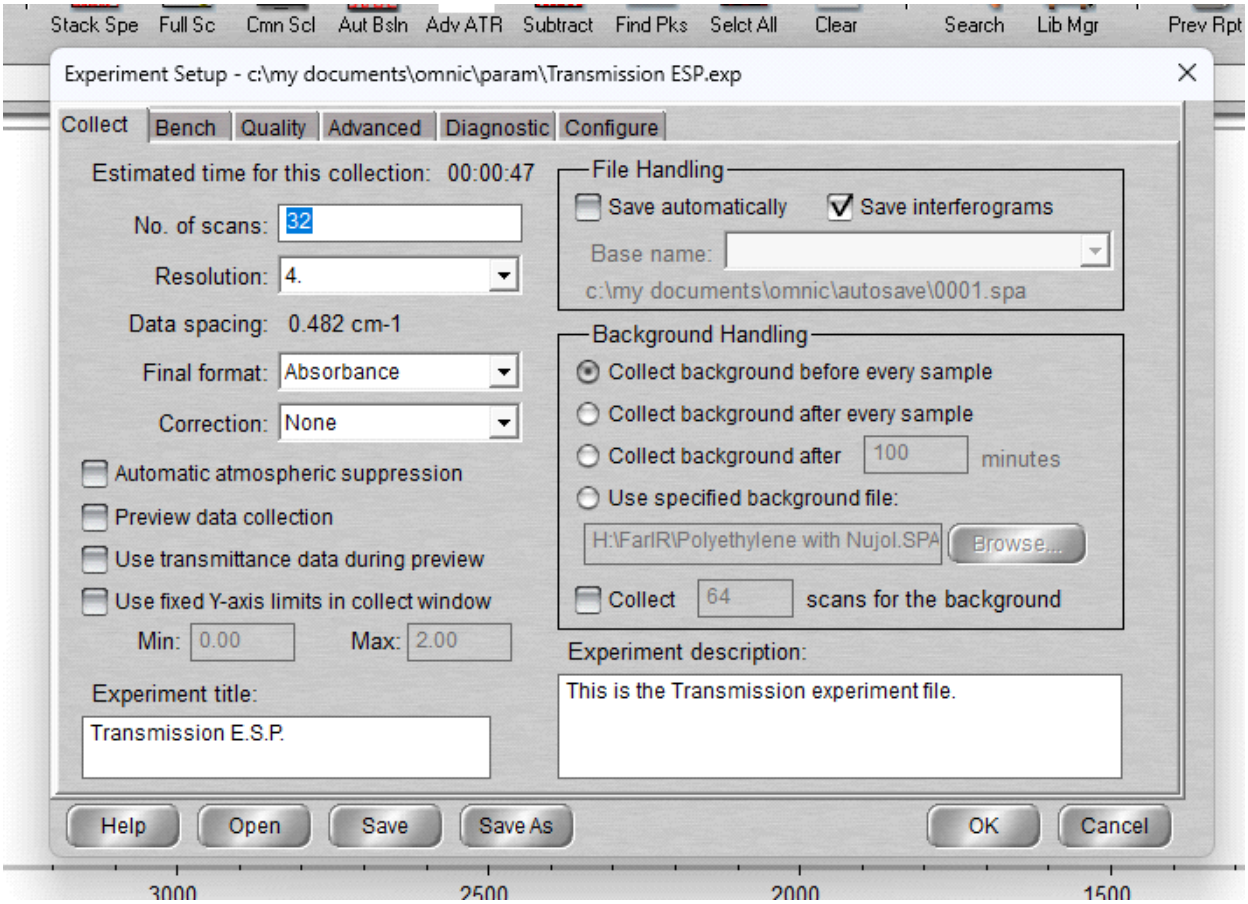

Figure 9. Experimental Setup Collect Tab

9. Input the No. of Scans, final format (recommended is “Absorbance”) and your background handling option (recommended one is "Collect background before every sample"). This option will run background check before every sample analysis. This is important to omit the signals from surrounding air in ATR mode. Make sure the experimental title shows ATR.
10. Click Bench> The red signal peak should be within two black borders (Fig. 10 is within range) and check the parameters on the right. Sample compartment (ATR), detector (ATR), Beamsplitter, Source (KBr), Accessory, window should be as displayed in Figure 10for default run. You can change the scan range here for your specific experiment (recommended range is 4000-350). Leave the Quality and Advanced settings on default.

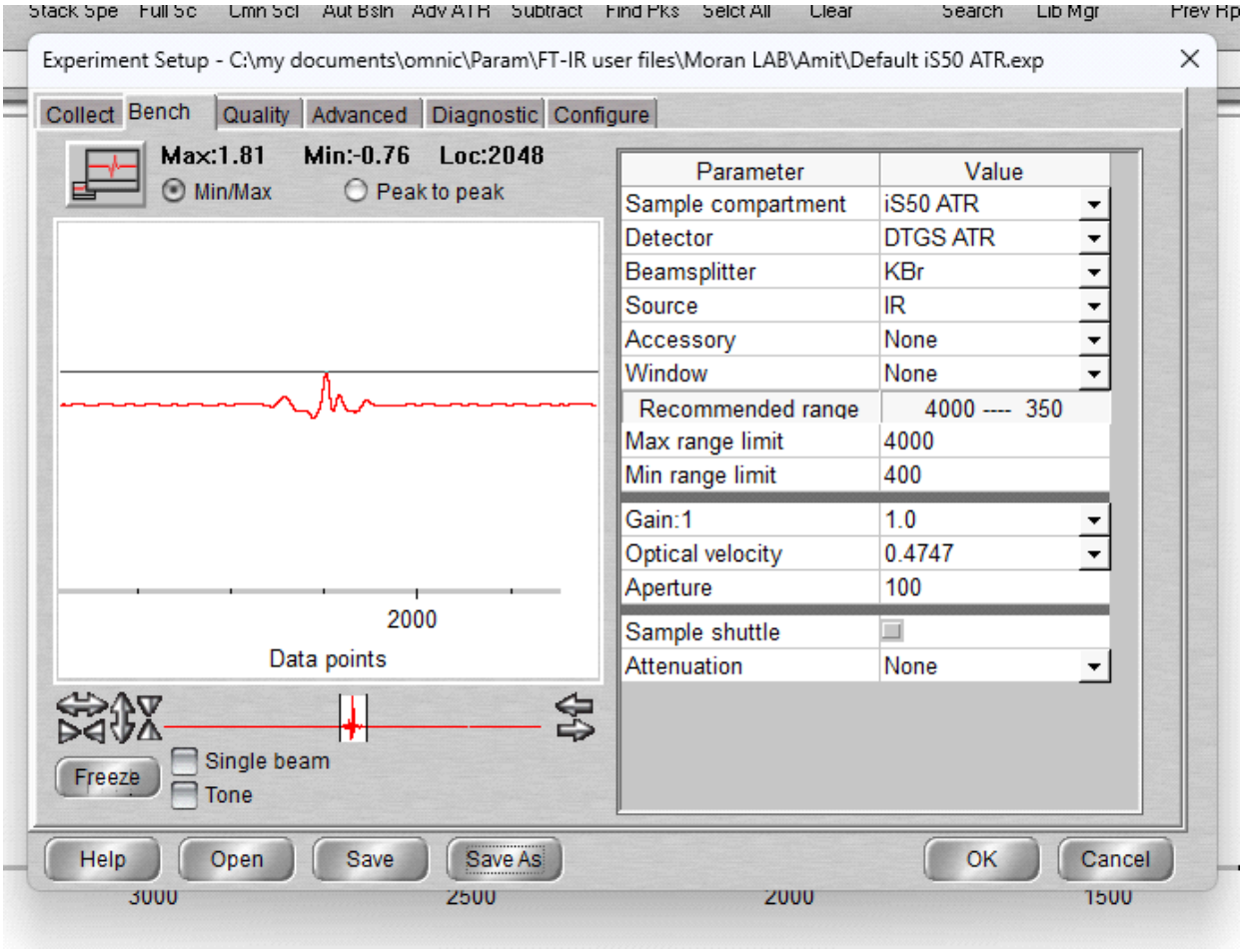

Figure 10. Experimental Setup Bench Tab

11. Go to Diagnostics, check the power, laser, and source by clicking the blue icons one by one, each time you will get a pop-up window with green ticks. If No errors, sample collection can be started! Click OK in all the pop-up tabs and return to the main panel for measurements.

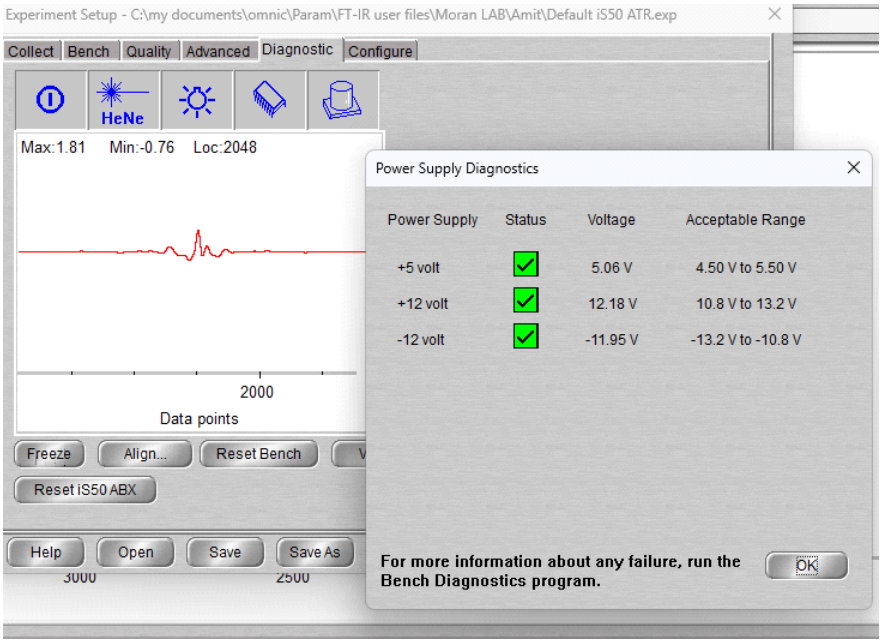

Figure 11. Power Supply Diagnostics

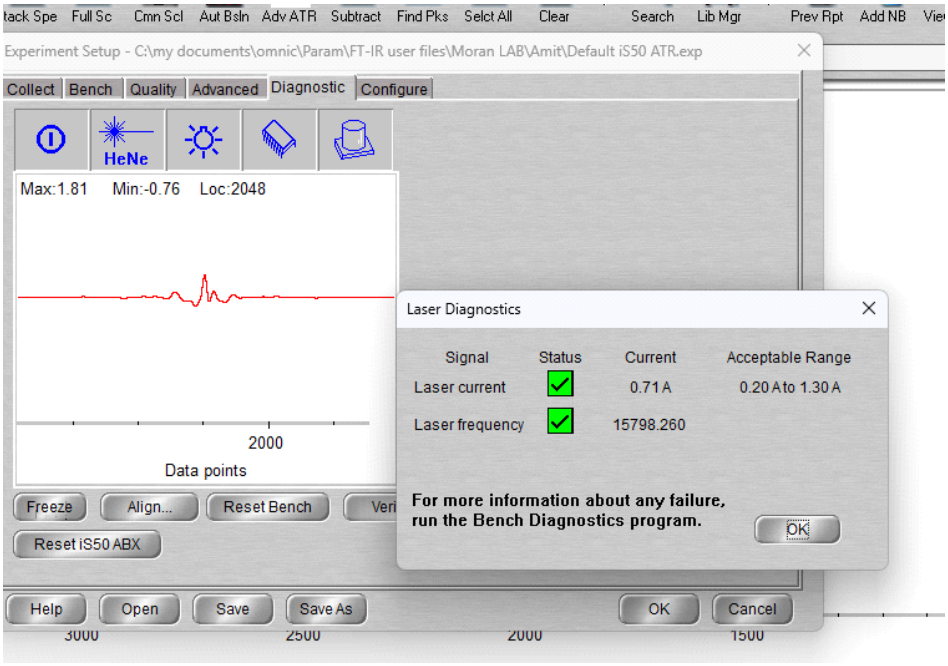

Figure 12. Laser Diagnostics

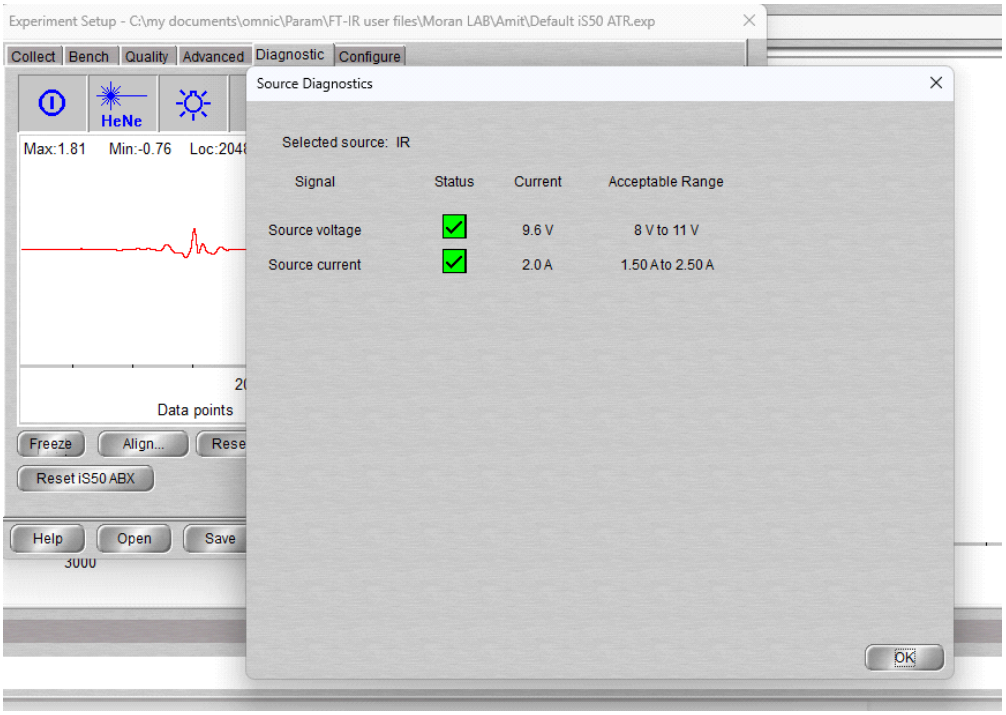

Figure 13. Source Diagnostics

4.4. Collecting Sample & Measurements

4.4.1 Collecting Background

12. For measurements – Click Collect Sample (Col Smp). Enter the spectrum title.

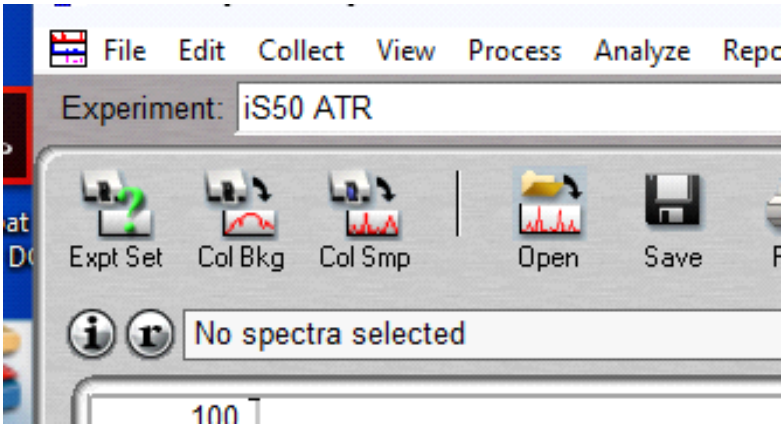

Figure 14. Experimental Setup Button

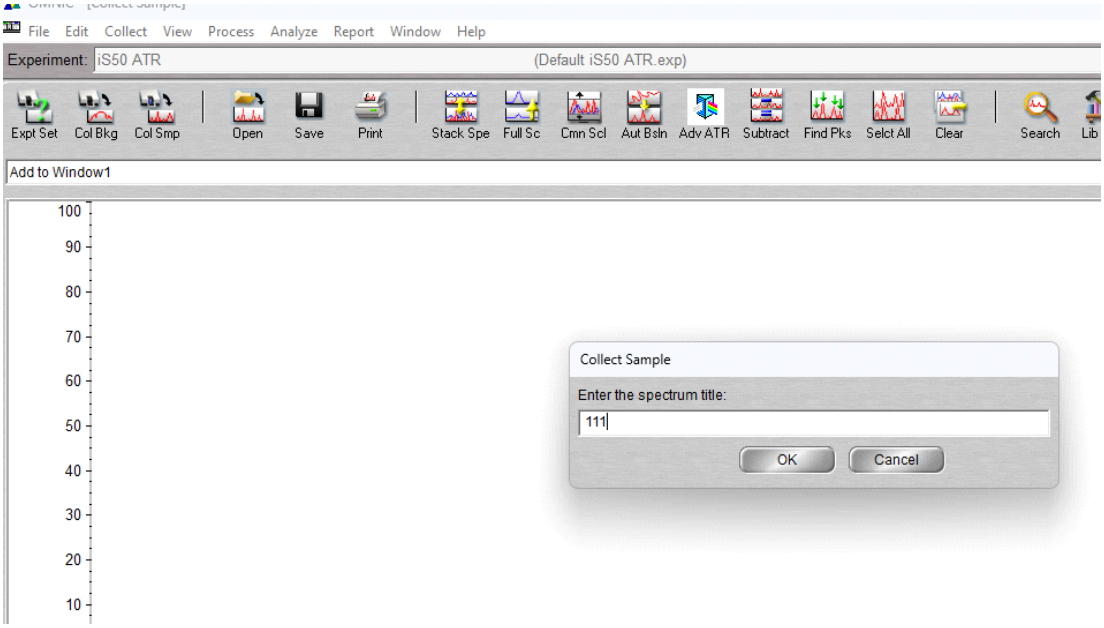

Figure 15. Spectrum Title Menu

13. As default, background collection before each sample was selected. Click “Ok” to get the background signal (in this case air, no sample). The background collection signal will look like Fig 17 below for air.

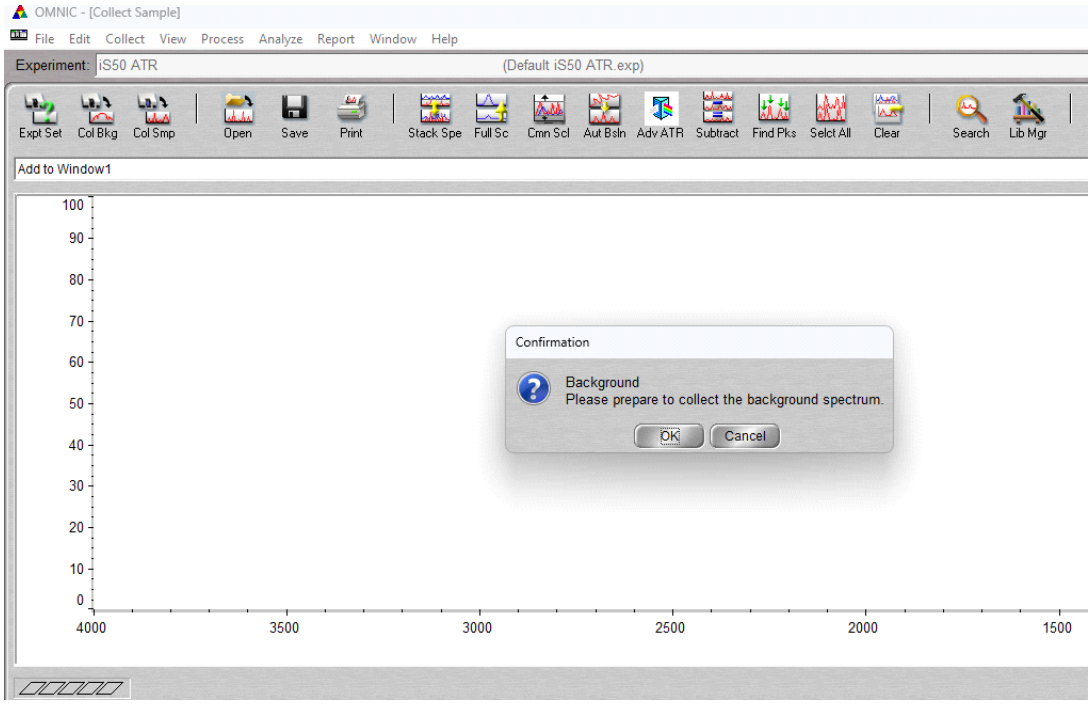

Figure 16. Background Sampling in progress

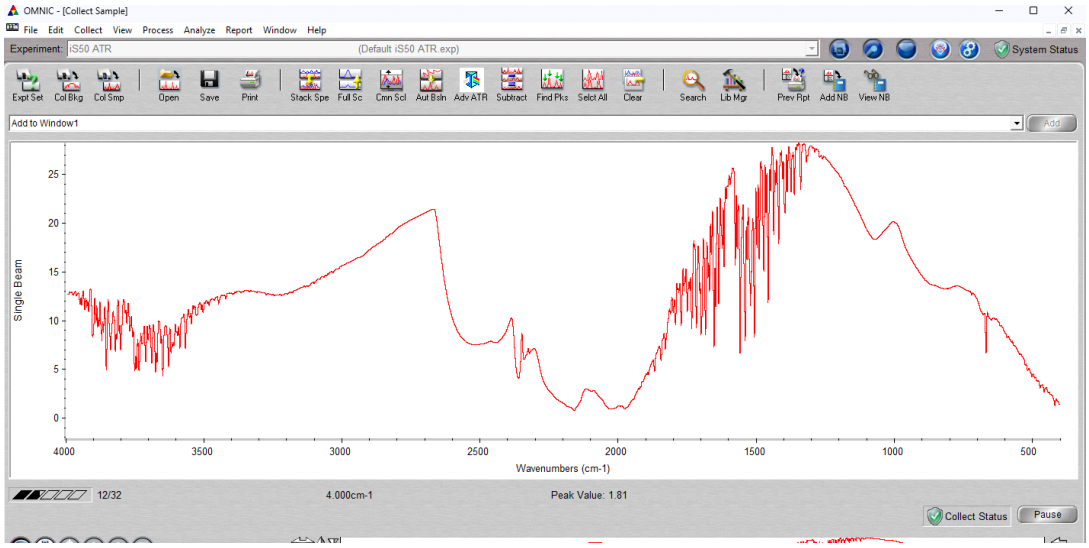

Figure 17. Background Sampling completion data

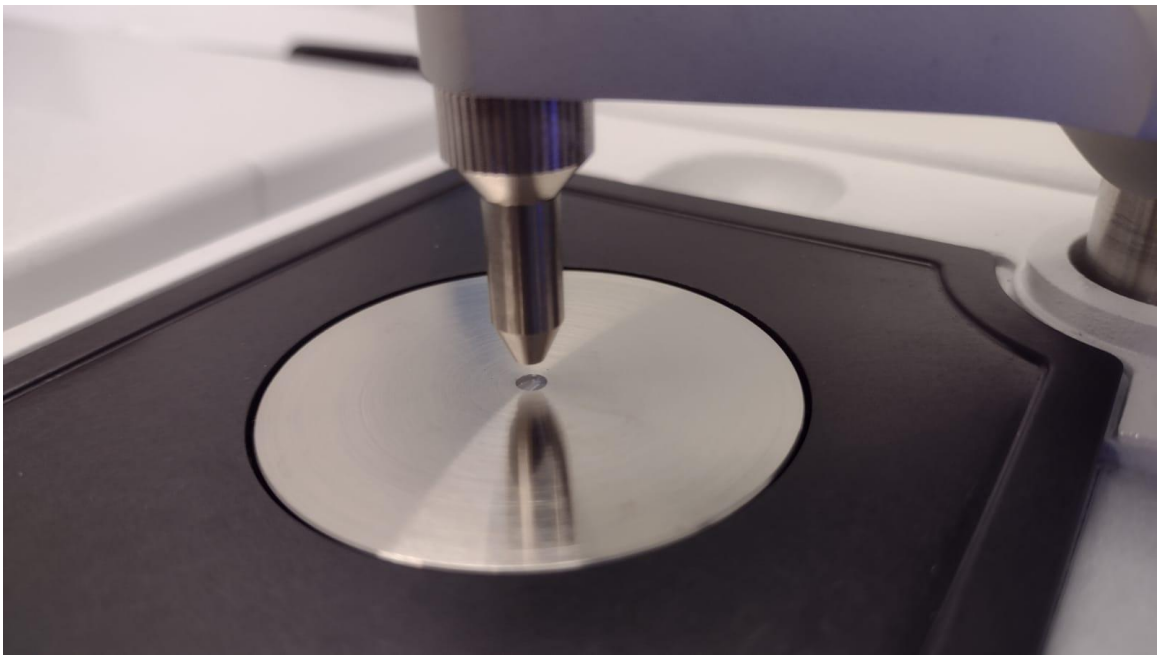

Figure 18. Example of ATR Background loading

4.4.2 Collecting Sample

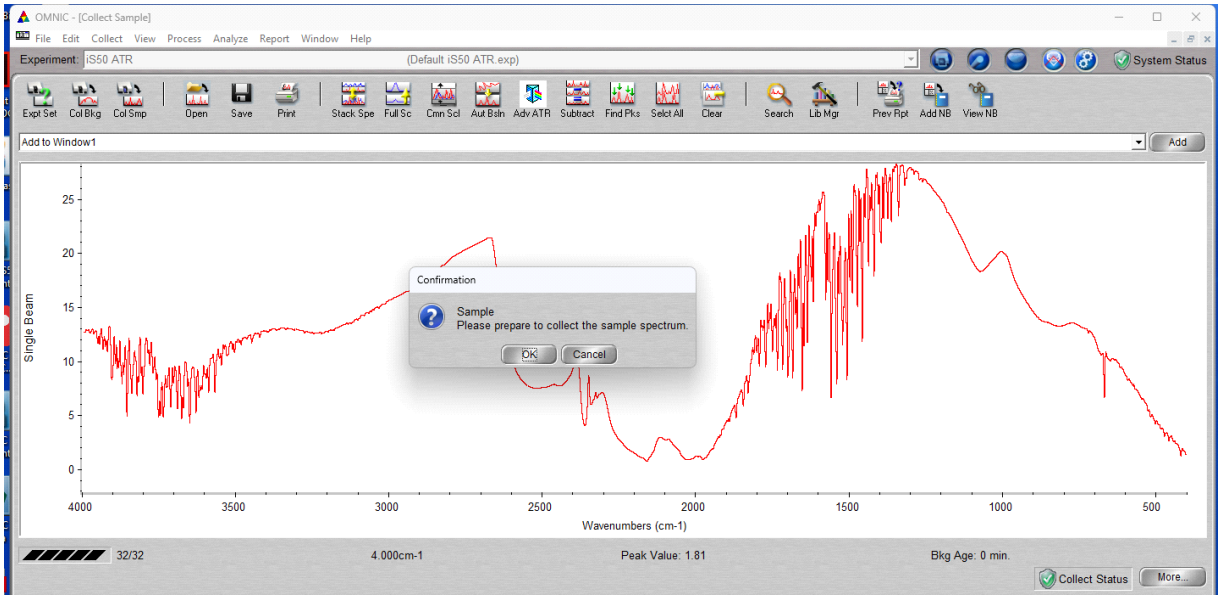

Figure 19. Pre-processing menu before sample collection

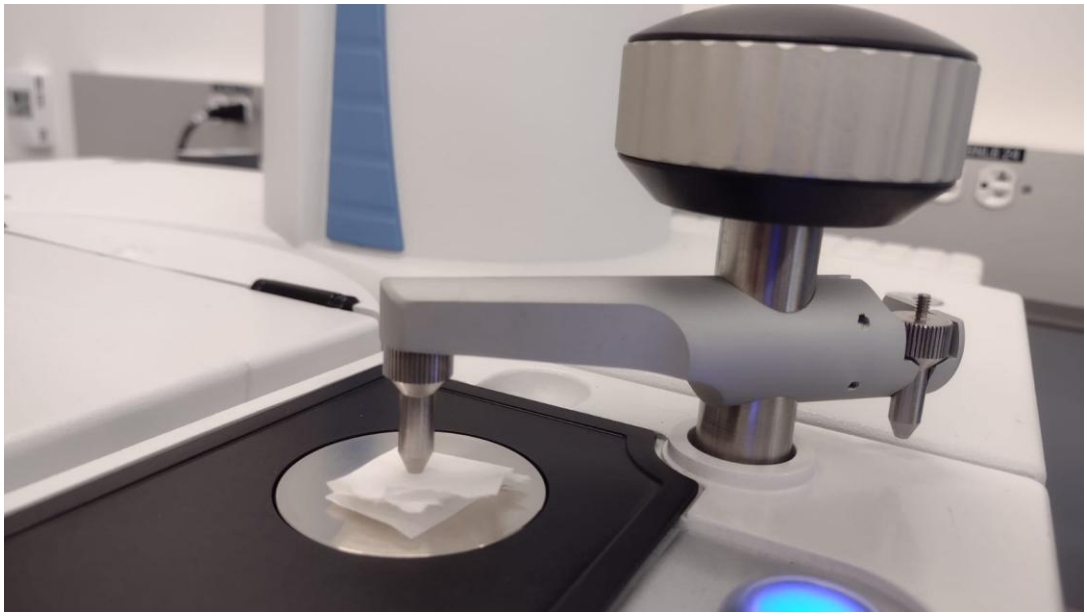

Figure 20. Example of ATR Sample loading

14. Once the background data is obtained, place the sample as in Fig 20. For powder samples, ensure the sample is tight between diamond crystal plate and the pressure tower. After sample is loaded, press OK on the pop-up to start the scan. The image below is the scan

signals from the lint-free tissue paper. After scan completion, a pop-up window will appear (image below), click OK. This will open a new window for post-processing.

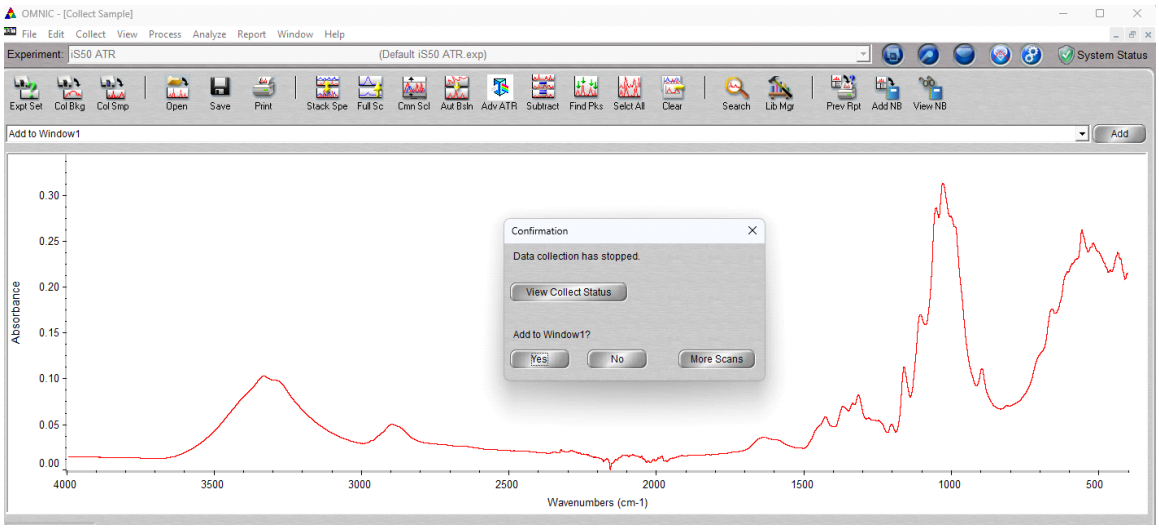

Figure 21. Post-Processing Window

4.5. Data Processing & Analysis

15. Fig 22 is absorbance spectra in ATR mode. To convert it into %Transmission, Go to PROCESS and select transmission from the drop-down menu.

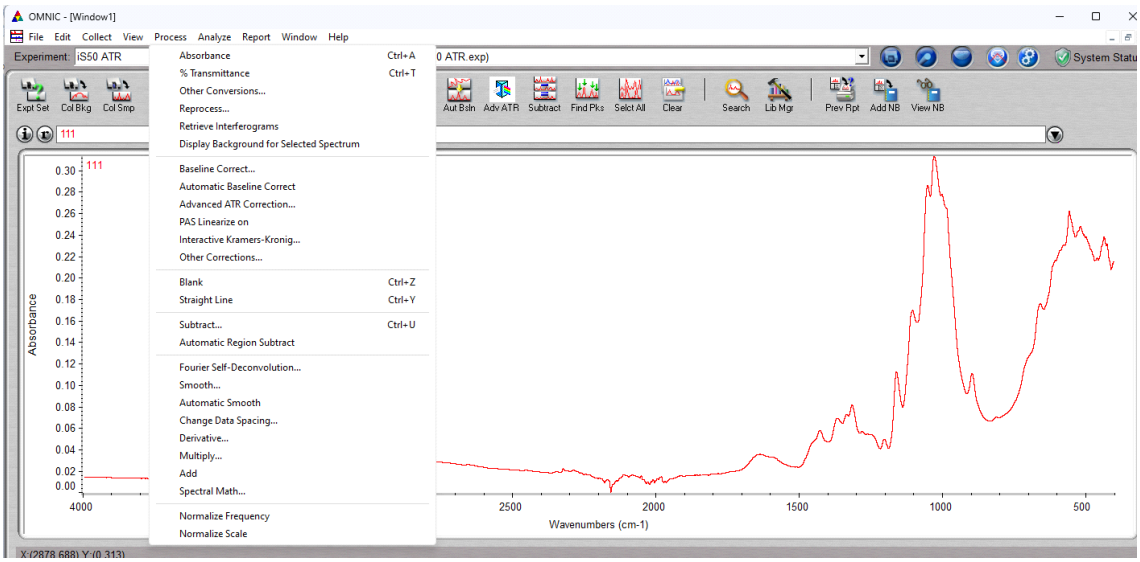

Figure 22. Process drop-down menu

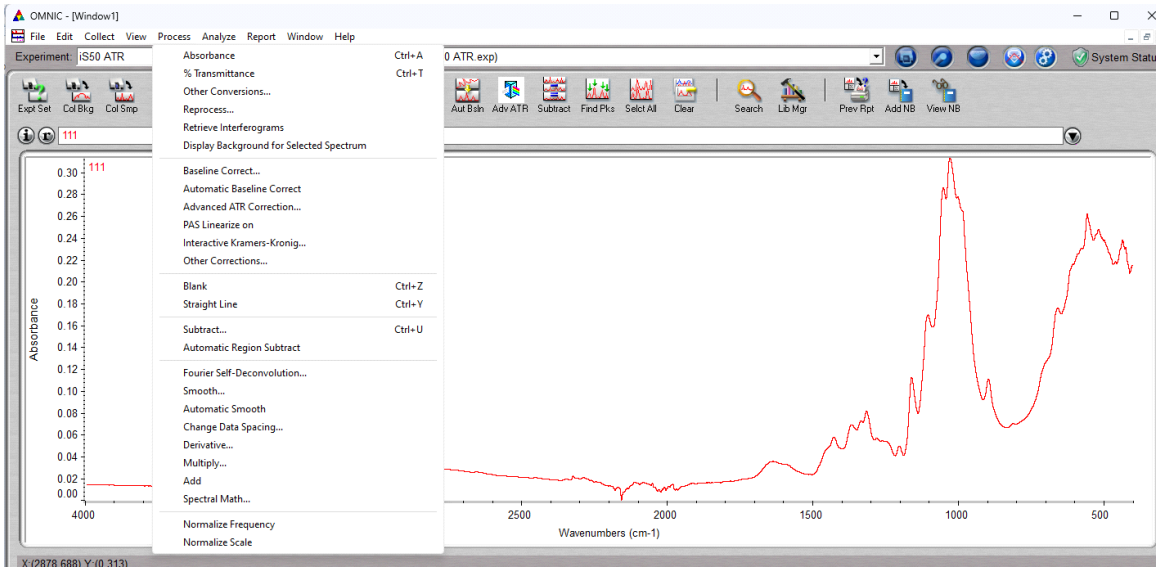

Figure 23. Transmission option

14. The transmission data will look like Fig 24.

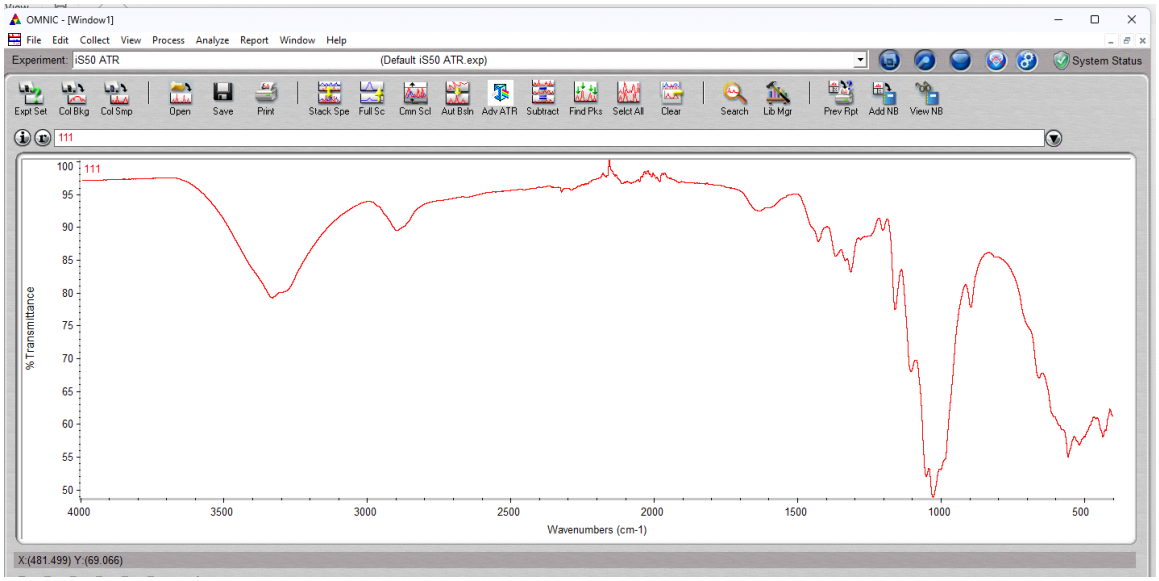

Figure 24. Transmission Data Graph

15. For post-processing (ATR correction, baseline correction) you need to get back to Absorbance mode from the drop-down menu. The selected spectra will be highlighted in RED, make sure to select the right spectra in case of deleting any spectra after multiple sample scans.
16. Analysis can be operated on %Transmission data. For Analysis, there is a basic library.

Click Analysis tab>library setup (a pop-up window appears)> Add the libraries from left to right (shift select all>Add).

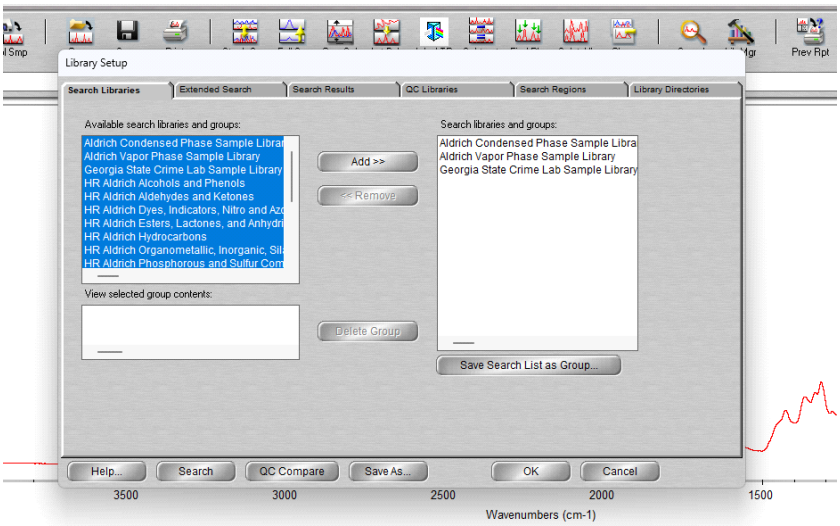

Figure 1: Selecting Libraries for analysis

17. After selection, click OK> Analysis> Search.

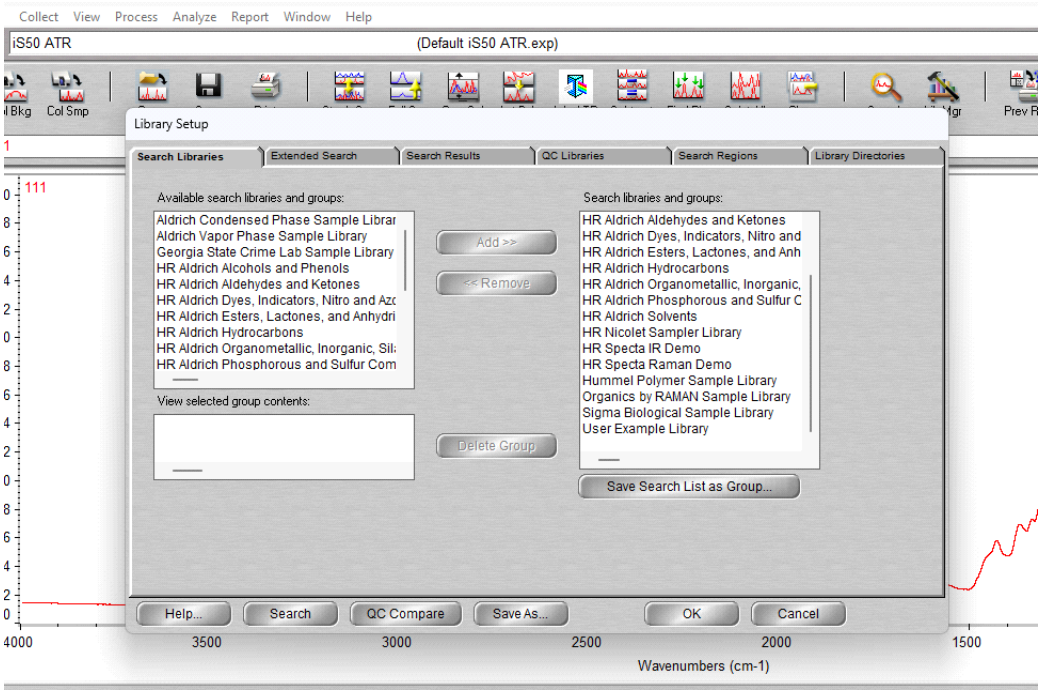

Figure 2: Selecting libraries for analysis

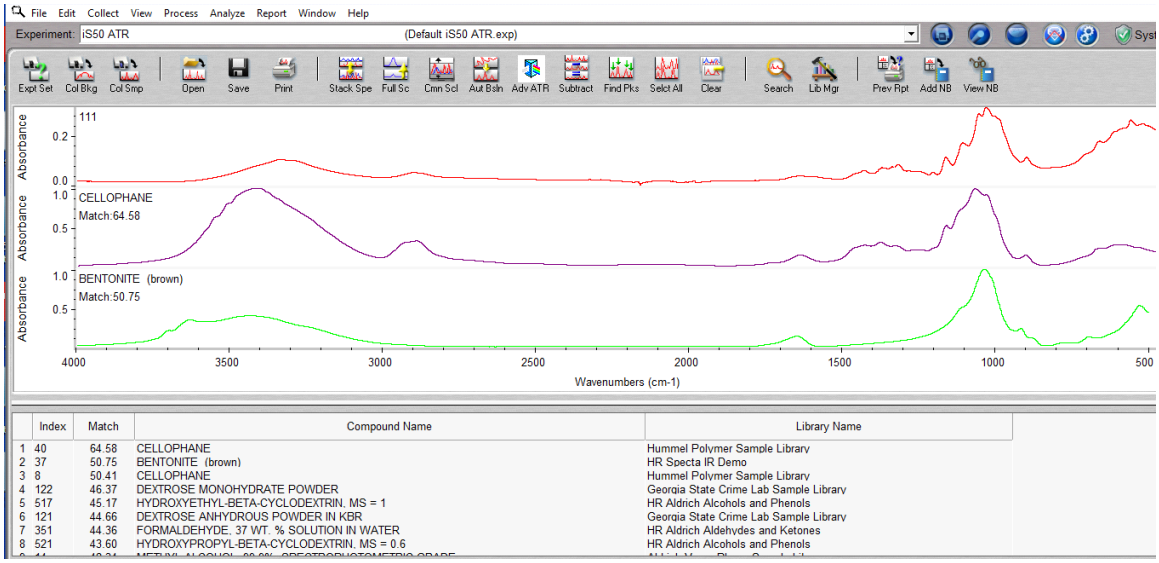

Figure 3: Library reference graph

18. The analysis operations like IR spectra identification, peak finder, etc. can be performed from drop-down menu with one-click.

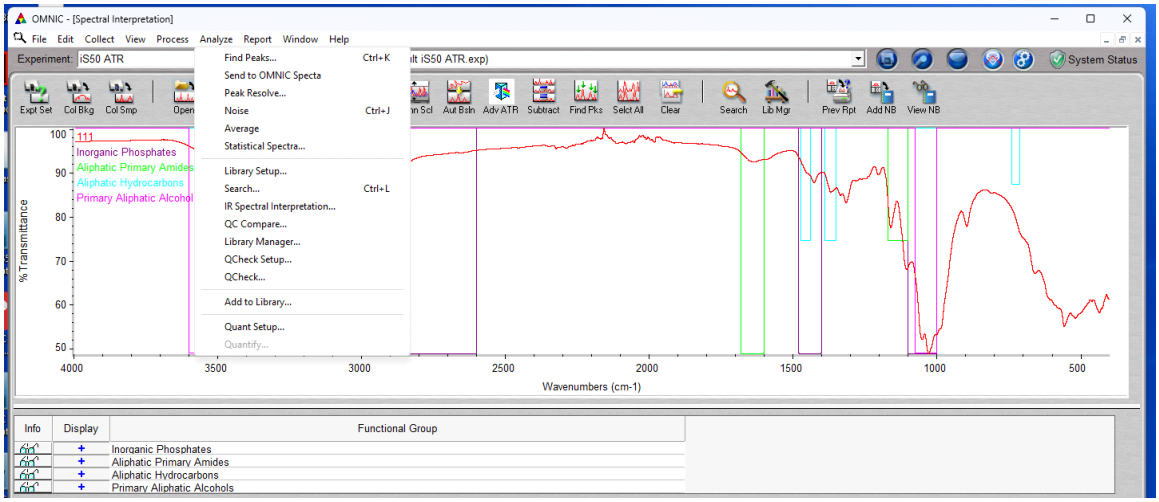

Figure 4: IR Spectral Interpretation Menu

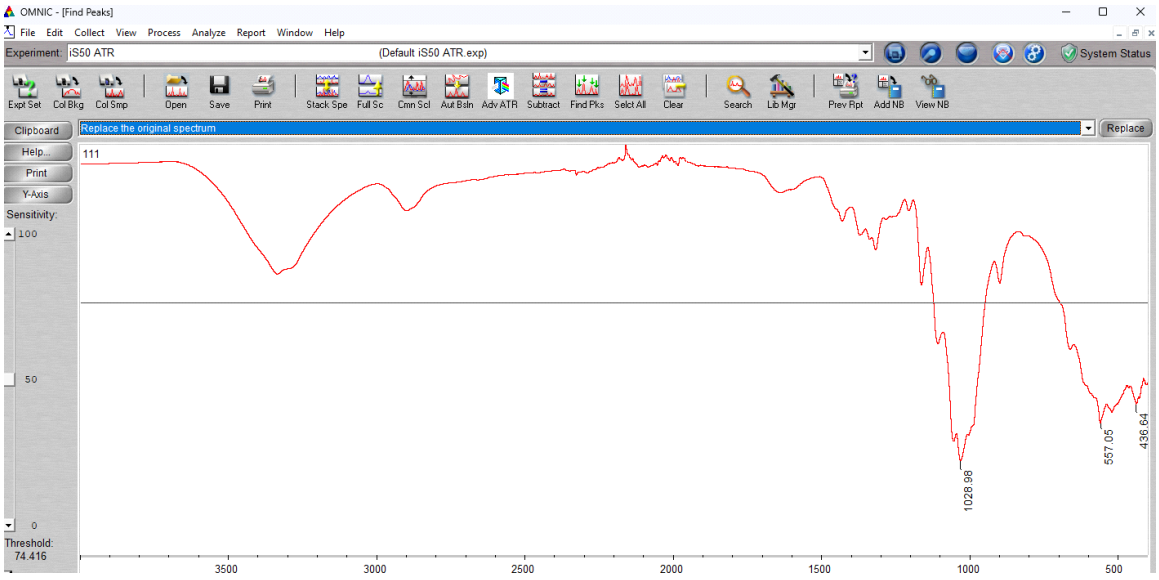

Figure **5**: Peak finder Menu

19. A report can be created by clicking Report>Preview/Print Report>a new pop-up window appears>Print>Microsoft Print to PDF> name the file and save it as PDF at your desired location.

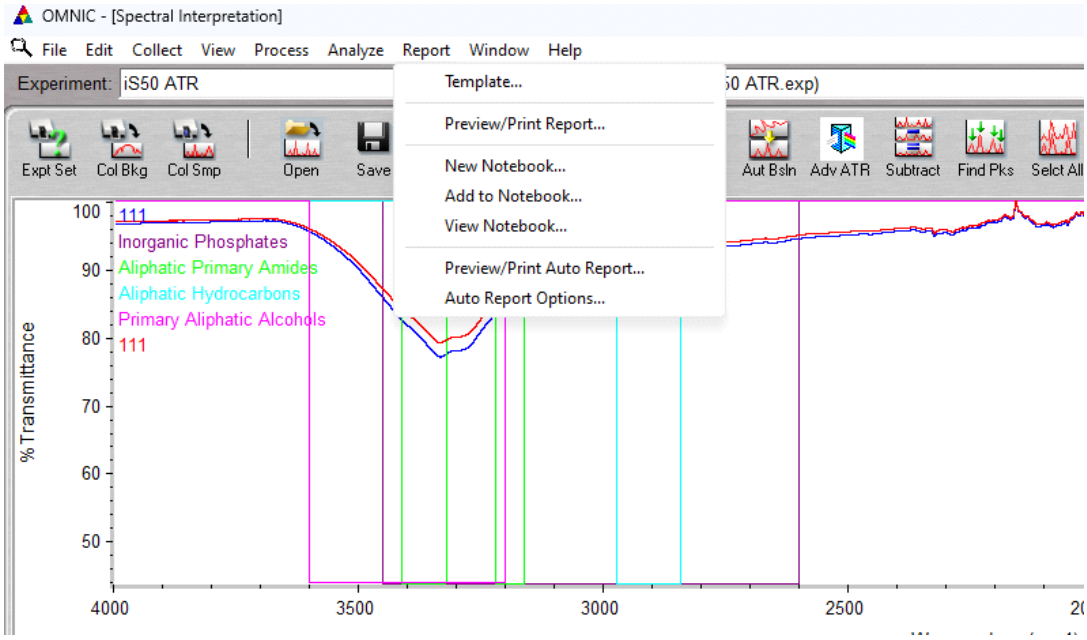

Figure 6: Report drop down menu

20. TO SAVE data: Go to File>Save as> Spectra > FTIR data users > create your lab folder and save it there. Do not save in Spectra folder as it contains standard data from library.

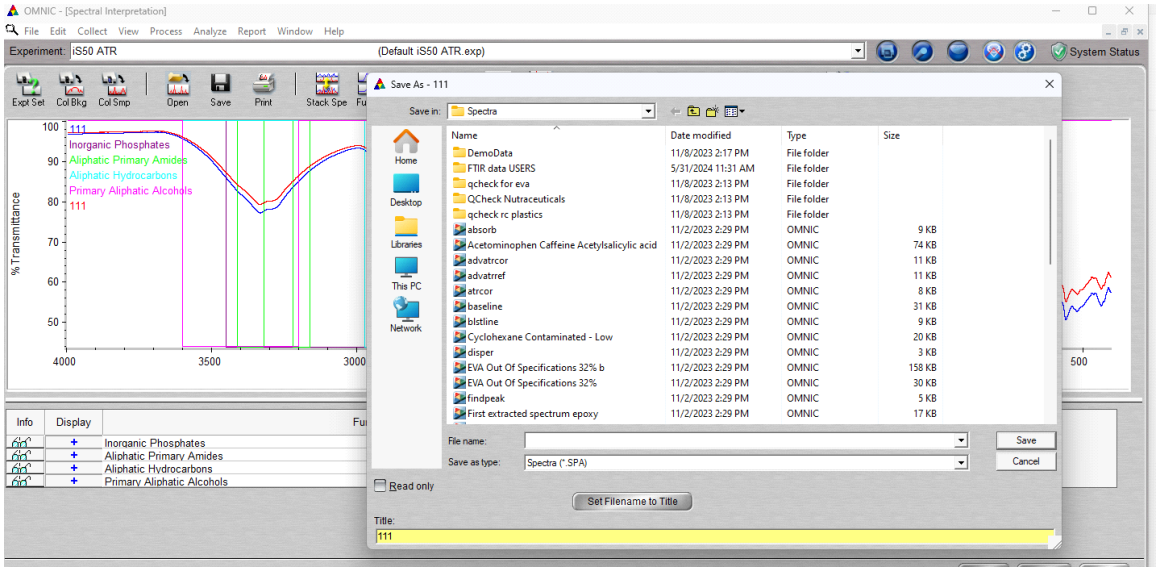

Figure 7: Saving to Pen Drive

21. The short-cut to the FTIR data user folder is also on the desktop for direct data access.

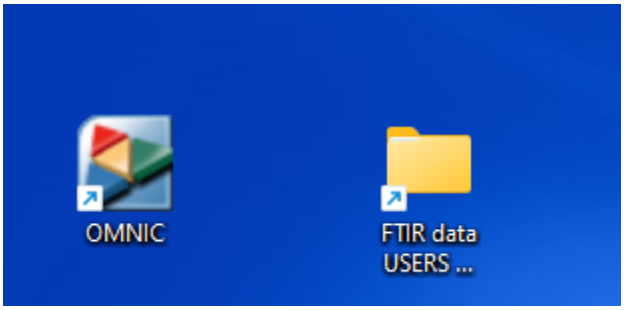

Figure 8: Starting home screen

4.6. Completion

23. Clean the sample from the analysis window properly using a lint-free tissue paper and minimal amount of isopropanol. Do not forget to clean both the diamond crystal plate and the pressure tower. Do not use sticky samples for analysis without consulting NFF staff to know the proper cleaning protocol for sticky samples like polymers and viscous liquids.