

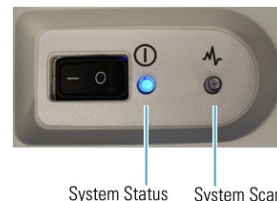
# OPT5: Operating Procedure for Transmission Data Thermo Fisher Nicolet iS50 FT-IR Spectrometer

*Refer to the OMNIC program help menus*

*Note: Right clicking on selections from the various pull-down menus in the software will bring up a help menu for that selection*

1. **Ensure you have an active OPT5 reservation.**

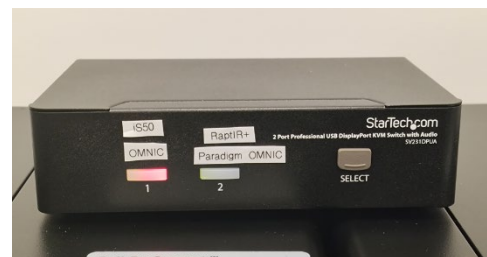
2. **Verify that the system is on.** There should be a blue power light on the back left of the instrument. If the light is any other color than blue, please do not use the instrument and notify SMIF staff. The system scan indicator may be flashing blue, which is acceptable.



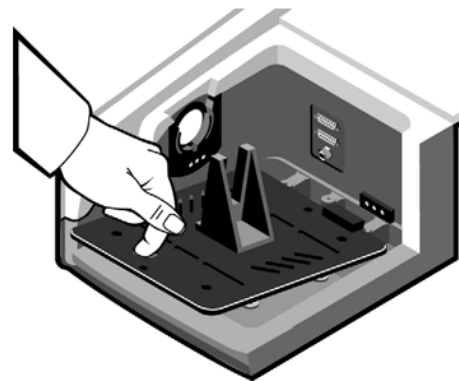
3. **Check the desiccant.** The round indicator on the desiccant compartment cap should be blue. If the indicator has turned pink or white, notify SMIF staff and do not use the instrument.



4. **Turn on the OMNIC computer.** The black box on top of the two instrument computers has a red light that indicates which computer is active. If the RaptIR+ computer is active, ensure that the Paradigm software is closed and press the gray “SELECT” button to switch to the computer for the iS50, which runs OMNIC software.



5. **Open the sample compartment cover at the front of the instrument.** Install the baseplate with standard sample holder into the sample compartment. Slide the baseplate back until it snaps into place and is held in place magnetically. Ensure there is good contact between the electrical connectors at the back of the baseplate.



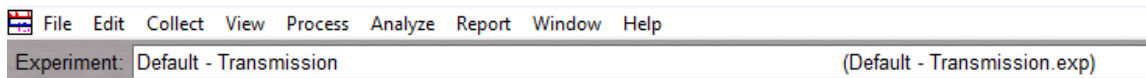
6. **Open the OMNIC software** on the computer by double clicking on the desk top icon. The system will automatically identify the sample compartment configuration that is being used. If the sample compartment **touch point** is not already lit solid blue, touch the touch point. It will flash blue for several seconds while the system reconfigures and remains lit when the system recognizes that the sample compartment is ready to be utilized.



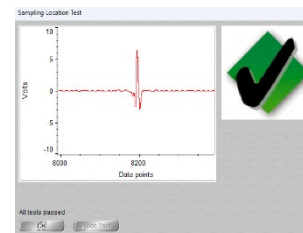
Sample Compartment  
Touch Point



7. **Load the default file.** For transmission, the file is “**Default – Transmission**”.



8. **The instrument will perform several self-checks.** Once the instrument shows an interferogram with a green check mark and states “All tests passed”, hit OK.



9. **Verify or modify the experiment settings** by clicking on the “Experiment Set-Up” icon in the top left (or by pressing Ctrl+E)

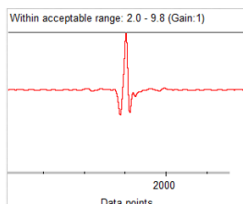
a. For an explanation of the settings, find “Experiment” in the Index of the OMNIC help topics

b. **Select the Collect tab and enter the desired values for:**

- i. **Number of Scans** (32 is typical, increasing this number reduces the noise level and makes smaller absorptions easier to distinguish)
- ii. **Resolution** (4 is typical and recommended)
- iii. **Final Format** (either %Transmittance or Absorbance is recommended)
  1. **%Transmittance** is used to compare the spectrum visually with published reference spectra
  2. **Absorbance** is used for quantitative analytical measurements / comparing spectra to commercial libraries
  3. **%Reflectance** is used for reflection techniques like ATR
  4. **Log (1/R)** units are for spectra collected using reflection techniques for quantitative comparisons
  5. Other final formats are not relevant to transmission
- iv. **Correction** (none)
- v. **Atmospheric Suppression** (For known samples, this is recommended. Atmospheric Suppression may mask peaks in unknown samples.)
- vi. **File Handling** (It is strongly recommended that you save interferograms. With this, you can restore original data after processing and keep an archive of original data.)
- vii. **Background Handling** (The default is to collect a background before every sample. For most applications, you don’t need to collect a new background spectrum for each sample spectrum if parameters have not changed.)

c. **Select the Bench tab**

- i. Verify that the laser interferogram is present
- ii. Verify that the value for “Accessory” is “Transmission E.S.P.”
- iii. **Set the desired Max. and Min. range limit** for the measurement (in wavenumbers). To change them, double click the value and type the desired value.
- iv. **Gain** amplifies the detector signal intensity and is helpful when the IR signal is weak. Autogain is suggested.
- v. **Optical Velocity** is the speed of the moving mirror in the interferometer. Slower velocities take longer to collect a spectrum and



increase the spectrum intensity. 0.4747 is the suggested optical velocity.

- vi. **Aperture** controls the size of the angular size of the IR beam and thus the amount of radiation that reaches the sample and the optical resolution. For the DLaTGS detector, the aperture size should be 100.



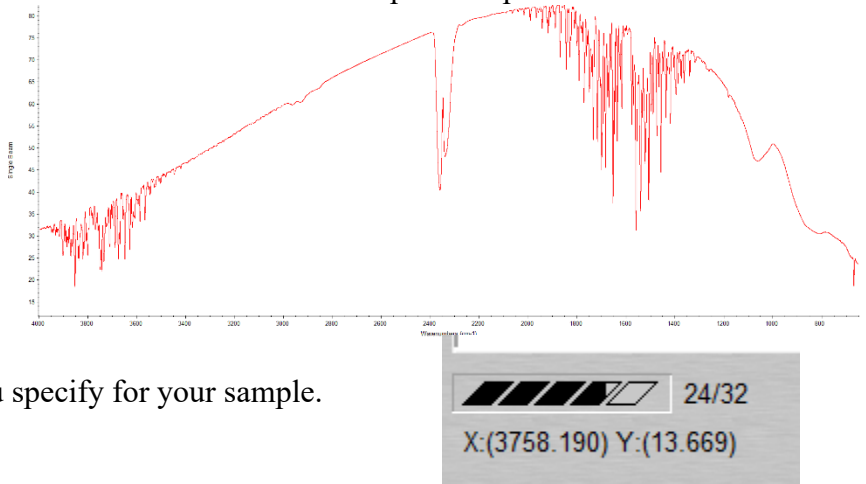
10. **Begin the experiment by pressing the “Collect Sample” button** in the toolbar.

- a. Alternatively, you can press the **touch point** next to the sample compartment.
- b. You will be prompted to **give a title to the spectrum**. Enter the title of the sample you plan to run. If you do not enter a title, the software will default to a name that details the date and time of the experiment. Hit OK or the **touch point** to move forward.



- c. **You will be prompted to collect the background. Open the sample compartment and ensure that there is no sample in the sample holder.** Once you have verified that no sample is present, hit OK or the **touch point**.
- d. A background spectrum will appear in the Collect Sample window. A typical background spectrum will look similar to this the spectrum pictured below.

While the spectrum is being collected, you can view the status of the scans in the lower left of the software. The amount of background scans will match the amount of scans you specify for your sample.



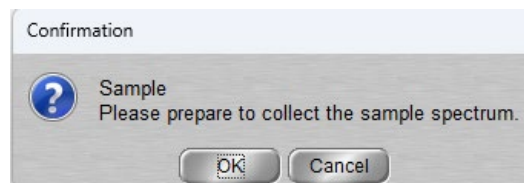
11. **Once the background scan has been completed, open the sample compartment and add your sample.**

The sample holder is designed for film samples, filters, and slide-mounted accessories. Slide the sample into one pair of slots in the sample holder. Ensure the sample is centered.

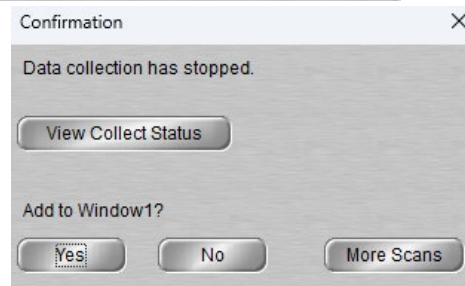
12. **Adjust the sample height if needed** to ensure the beam path passes through the sample. The beam is centered approximately 3.5 inches above the baseplate. Turn the adjustment screw at the bottom of the sample holder: counterclockwise turns move the sample up; clockwise turns move the sample down.



13. **When you are ready to scan your sample, hit ‘OK’ or press the touch point.** As data is collected, the sample spectrum in the Collect Sample window is updated. The number of scans collected and the total number of scans for the collection are displayed to the right of the gauge at the bottom left of the screen.



14. Once the scan is complete, you will be prompted to add the spectrum to the viewing window. **Choose “Yes” to add the sample spectrum to the window.**



15. If needed, click the spectrum to select it. The active spectrum will be red. **To save the file, choose File > Save As and enter a file name.**
- Choose your personal directory within the UserData folder on the desktop for the file location.
  - Select the file type. The most common file types to save are:
    - Spectra file (.SPA) (OMNIC format, includes experimental conditions and interferogram / raw data)
    - Text file (.CSV)
    - Image file (.TIF)
  - The experiment conditions under which the spectrum was collected can be viewed by clicking the “i” information button beside the spectrum list pull-down menu. These conditions can be saved by clicking the copy button and pasting into the Notepad program, then saving as a text file.



16. **Process the data.** (If desired.)

- The OMNIC software can be used to convert the spectrum units, find and label peaks, adjust the spectrum layout, and overlay or compare spectra using commands under the View, Process, and Analyze pull-down menus.
- Right click on any of the commands in these menus to view the online help file for that command.
- The command “IR Spectral Interpretation” in the Analyze pull-down menu can be useful for identifying various chemical (functional) groups in your sample.

17. **Compare the spectrum to known materials in the library.** (If desired.)

- Select the desired spectrum by clicking on it. The active spectrum will be red.
- Choose “Library Set-Up” from the Analyze pull-down menu.
- Choose the libraries for your search and then click on “search” to produce a set of matches.

18. **When finished:**

- Unload your sample.
- Remove the baseplate by inserting your finger into the large hole near the front of the baseplate and lifting the baseplate out of the compartment.
- Exit the OMNIC software.
- End your active OPT5 reservation.
- Leave the FT-IR system power on.**