

Standard Operating Procedure

Task: Thermo Nicolet iS5 IR Spectrometer Operation

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Background:

- Infrared (IR) spectroscopy is a fundamental analytical technique in synthetic chemistry. The Nicolet iS5 is designed for analysis of vibrational features in solution or in thin films. For in situ spectroscopic reaction monitoring by IR, consider using one of the ReactIR instruments available in the department.
- IR spectra can be acquired in either (A) attenuated total reflectance (ATR) mode or (B) transmission mode. The ATR mode is used to interrogate thin films (liquid or solid) layered on a solid crystal surface. The IR beam is reflected along the backside of the crystal, which leads to an “evanescent wave” that interacts with the analyte. The transmission mode is used to interrogate solids (in a salt pellet or mulled on salt crystals) or solutions (with the analyte dissolved in a solvent using a between salt plates in a specially designed analysis cell). The IR beam is directed through the sample.
- Choose the appropriate mode for IR spectroscopy. If you are working with materials that are stable to air and moisture, the ATR accessory is quite convenient. If you are working with materials air- and moisture-sensitive materials, the solution cell is a good option. If needed, the ATR accessory can be pumped into the glovebox and the sample prepared under N₂. Once the anvil has been closed on the sample, an o-ring seal protects the sample.

Training Requirements:

- Lab safety training
- IR training
- Glovebox training

Potential Hazards:

- Optical components in the spectrometer are sensitive to dust and moisture and need to be covered at all times unless changing the accessory. There is a paper humidity indicator on the back of the instrument. The desiccant needs to be changed when the paper indicator changes from blue to pink.
- The ATR crystal is sensitive to pH and some solvents. Use care when preparing liquid samples and when cleaning the cell
- This instrument is a laser product with an 850 nm diode laser.

Special PPE Requirements:

- Standard PPE

Materials Needed:

- Prepared solid or liquid sample in the ATR crystal or the liquid holding cell.
- Acetone and Kimwipes for cleaning (ATR)

- Syringe for sample loading (ATR or solution)

Procedure:

Switching between accessories (ATR versus solution iD1 Transmission Accessory)

- Each accessory is readily interchangeable. When not using an accessory it is generally stored in the a small white box behind the Nicolet instrument
- To change the accessory, remove the current one from the Nicolet iS5. Insert the desired accessory in its place so that it lines up with the magnets on the Nicolet instrument and snaps into place. When inserting a new accessory a new window will pop entitled 'smart accessor change' where you need to choose the appropriate experiment
 - For ATR: Default – Transmission (default.exp)
 - For iD1 Transmission: iD1 Transmission (iD1_Transmission.exp)

Using the ATR Accessory: Germanium Crystal

Preparing liquid samples with the ATR accessory

- Ensure that your sample will not react with the crystal material. Applying extremely hot or cold liquids may crack the crystal.
- Liquid samples are simply poured or injected directly onto the ATR crystal. Sample may also be applied if dissolved in a volatile solvent. Use a syringe needle to add small droplets to the ATR crystal (Note: *do not scratch ATR surface with needle*), wait until it evaporates. Continue adding droplets until a visible layer has been added.

Preparing powder samples with the ATR accessory

- Sprinkle a thick (1-3 mm) layer of the sample directly on the crystal, making sure the crystal is completely covered with the powder. A powder press is typically used to achieve even distribution and contact with the crystal.
- Do not use the pressure device on the ATR accessory to crush a sample, use a mortar and pestle if necessary.
- Uneven samples can result in tilted baselines in the IR spectrum.

Collecting a Sample with the ATR accessory

- To change the parameters of the experiment go to Collect→Experiment Setup or click the "Expt Set" hot button from the toolbar. Parameters you may want to adjust are listed below. If you wish to create a new experiment that can be selected from the experiment drop down menu, save the experiment with a new title, otherwise simply click "OK"
 - Number of Scans: 32 is recommended for ATR experiments. The greater the number of scans, the greater the signal to noise ratio but the longer the experiment will take.
 - Resolution: usually 4 cm^{-1} . For liquid samples, bands are often broader and a lower resolution (8 cm) may be required. Powders tend to product finer features than solids and may require a higher resolution setting (2 cm). If you increase the resolution, you may also need to increase the number of scans since data collection at a higher resolution will contain more spectral noise.
 - Background handling: The background collection is set to collect a new background every 500 minutes (~ 8 hours), if you wish to take a new background before every sample, you can set that parameter here. *Note: Changing any parameters in the experimental setup will require the acquiring of a new*

background sample before a new sample under different experimental conditions.

- Collect a Background
 - Ensure there is no sample on the ATR crystal and that it is open to the air.
 - Click the “Col Bkg” button on the toolbar.
 - Click “OK”
 - The background is automatically saved. Add the background to the window if desired. If you wish to view the background later, go to Collect→Display Spectral Quality Reference
 - Collect a new background if you see any changes in the spectral baseline or any of the Collect parameters have been changed.
- Collect a Sample
 - Click the “Col Smp” button on the toolbar
 - Enter a unique title or use the one automatically provided. Different samples can be selected from the title drop down menu.
 - Apply the liquid or powder sample to the ATR crystal. Apply pressure with the ATR accessory for powder samples. No pressure is required for liquid samples. If the solution is evaporated, lower the arm.
 - Click “OK”
 - After sample collection is completed, add the spectrum to the window
- Cleaning the ATR crystal
 - Unscrew the anvil head but take care not unscrew it completely as it can become stuck.
 - Remove the sample immediately after you finish the analysis.
 - Clean the crystal with water or with a suitable solvent (acetone, isopropanol). The crystal must be cleaned gently using a Kimwipe. Also make sure to clean the bottom side of the anvil as the sample can be transferred.
 - Dry the crystal with a Kimwipe

	Germanium	Diamond
Refractive Index at 1000 cm	4.0	2.4
Density (g/cm)	5.32	3.51
Hardness (Knoop #)	1150	7000
pH range	1-14	1-14
Physical Characteristics	Hard, brittle, temperature sensitive	Hard
Cleaning Solvents	Alcohols, acetone, water, toluene	Alcohols, acetone, water, toluene
Solvents to avoid – Attacks Crystal	Hot sulfuric acid, aqua regia	Concentrated sulfuric acid, K ₂ Cr ₂ O ₇

Pumping the ATR Accessory into the Glovebox

- First, follow normal procedures for obtaining a background spectrum with the ATR on the instrument. The background spectrum will be good for 500 minutes (~8 hours). If you need longer time this can be changed by going to > Collect > Experimental Set Up
- The ATR accessory will need to be pumped in through the large antechamber protected as best as possible from dust and particles
- Cover the ATR accessory in a loose bundle of aluminum foil to protect it from dust but leaving enough small openings to properly pump down under vacuum
- Bring into the glovebox, load sample onto ATR as previously described and lower anvil.
- Remove ATR from glovebox, replace on the Nicolet Accessory and follow standard sample collection and cleaning procedures

Using the iD1 Transmission Accessory

Preparing a sample in the liquid cell and iD1 transmission accessory

- Prepare an approximate 5-10 mM solution of your sample
- If you are taking an IR in an IR active solvent, a solvent blank must be taken first in the exact same solvent used to make your sample.
- The solution cell can be pumped into the glovebox. Air- and moisture-sensitive samples should be prepared in the glovebox and analyzed in transmission mode. The solution cell has Teflon plugs that form a seal with the metal housing that is sufficient for sensitive samples over short periods. Make sure to take your solvent background spectrum with the same solvent that is in your sample (ie degassed and from the solvent system)
- Two different solution cells are available. A 1.0 mm pathlength fixed cell and a demountable cell with different spaces (0.015, 0.025, 0.050, 0.1, 0.2, 0.5, and 1.0 mm).
- The cell will typically have a 0.1 mm pathlength, start with this and the spacers can be changed if necessary depending on the spectrum.
- When changing out the spacers be careful not to tighten accessory too much as the CaF_2 window can break.

Collecting a sample with the liquid cell accessory and iD1 transmission accessory

- The iD1 transmission accessory is a SMART accessory that, when installed, will automatically select the "iD1_Transmission.exp"
- To change the parameters of the experiment go to Collect→Experiment Setup or click the "Expt Set" hot button from the toolbar. Parameters you may want to adjust are listed below. If you wish to create a new experiment that can be selected from the experiment drop down menu, save the experiment with a new title, otherwise simply click "OK"
 - Number of Scans: usually 16 or 32. The greater the number of scans, the greater the signal to noise ratio but the longer the experiment will take.
 - Resolution: usually 4. The lower the number the more narrow the bands distinguishable.
 - Background handling: The background collection is set to collect a new background every 500 minutes, if you wish to take a new background before every sample, you can set that parameter here.
- Collect a Background
 - Make sure no sample is in the iD1 Transmission accessory.
 - Click the "Col Bkg" button on the toolbar.

- Click “OK”
 - The background is automatically saved. Add the background to the window if desired. If you wish to view the background later, go to Collect→Display Spectral Quality Reference
 - Collect a new background if you see any changes in the spectral baseline or any of the Collect parameters have been changed.
- If using a solvent that will require a solvent subtraction first collect a sample of just the solvent following the same procedure as below.
- Collect a Sample
 - Click the “Col Smp” button on the toolbar
 - Enter a unique title or use the one automatically provided.
 - Install the liquid cell between the two arrows in the transmission accessory and close the cover.
 - Click “OK”
 - After sample collection is completed, add the spectrum to the window
- When sample is completed, clean the IR cell with solvent used to take the spectrum, followed by acetone. Allow to fully dry before storing away.

Analyzing and Saving IR Spectra

- Typical workup procedures include solvent subtraction, baseline correct, smoothing the lines, and converting to % transmittance
 - Baseline correct: select ‘Process’ from the tool bar, then baseline correct. Select which method you wish to use. In the top window, click along the spectrum to drag the gray line to it matches up with the spectrum. Click “replace” in the upper right hand corner to complete the background subtraction.
 - If taking solution sample you can also perform a background subtraction by highlighting the two spectra you wish to subtract and selecting “Subtract” under the process tab. The bar on the left side of the window will allow you to change the level of subtraction until the desired spectrum is achieved
 - Smooth: Under process tool, click “Smooth” and typically the 12 cm⁻¹ option is used. This will add a new spectrum to the window denoted with an asterisk (*) as the edited spectrum.
 - Under process tab, click % Transmittance which will shift alter the spectrum to a typical IR spectrum.
- Use the buttons on the toolbar to pick peaks, change final format, and perform spectral math. See the “guide to the toolbar” below.
 - To peak pick select the “Find Pks” button and adjust the window in which peaks are picked. When satisfied, click “replace” on the right hand side of the window. Or using the “analyze” drop down menu and “Find Peaks”
- Perform library searches by adding or creating libraries in Analyze→Library Setup and using the search buttons on the toolbar.
- Find heights and areas using the analysis buttons in the bottom left of the OMNIC window.
- **Optional:** To create a report for the spectrum, go to Report→Template.
 - Select one of the pre-made templates and click “Select” or click “Create” to make your own. Add report Items to the window.
 - If a new report style was created, click “save as” and give it a title.

- Click the “Prev Rpt” button on the toolbar to view the report. You can print the report from here.
 - If any peak values are cut off, click close and zoom out with the tools in the bottom left of the OMNIC window.
- To save the spectrum, go to File→Save As. You can save the file as a .SPA file to reopen and edit in OMNIC. Save the file in your group drive, not in the lab notebook. You can also export the data as a .CSV file.

Related SOPs

- Glovebox SOP

Guide to the Toolbar



- Information Button: Gives information about the spectrum selected.



- Experiment Setup: Create specific experiments that can be later selected from the dropdown window. Parameters of note: No. of scans, resolution, final format, correction (for water, carbon dioxide, or specific reference spectrum), experiment title.

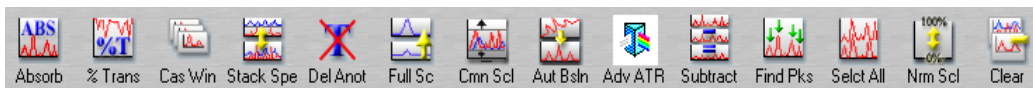
The screenshot shows the 'Experiment Setup' dialog box with the 'Bench' tab selected. The title bar reads 'Experiment Setup - c:\my documents\omnic\param\iD1_Transmission.exp'. The 'Collect' tab is also visible. The 'Estimated time for this collection' is 00:00:23. The 'No. of scans' is set to 16, 'Resolution' to 4, 'Data spacing' to 0.482 cm⁻¹, 'Final format' to Absorbance, and 'Correction' to None. There are checkboxes for 'Automatic atmospheric suppression', 'Preview data collection', 'Use transmittance data during preview', and 'Use fixed Y-axis limits in collect window'. The Y-axis limits are Min: 0.00 and Max: 2.00. The 'Experiment title' is 'iD1 Transmission'. The 'File Handling' section has 'Save automatically' unchecked and 'Save interferograms' checked, with a 'Base name' dropdown showing 'c:\my documents\omnic\autosave\0001.spa'. The 'Background Handling' section has three radio buttons: 'Collect background before every sample' (unchecked), 'Collect background after every sample' (unchecked), and 'Collect background after 120 minutes' (checked). There is a 'Use specified background file' option with a 'Browse...' button. The 'Collect 64 scans for the background' checkbox is checked. The 'Experiment description' is 'iD1 Transmission Accessory'. At the bottom are buttons for 'Help', 'Open', 'Save', 'Save As', 'OK', and 'Cancel'.



- Collect Background: Collect a background spectrum



- Collect Sample: Collect a sample spectrum based on the experimental procedure selected from the drop down menu.



- Spectral Editing Tools: Switching to and from absorbance and % transmittance units, spectral math tools, peak picking, etc.
- Search: Compare spectrum with those in the libraries selected through the library manager.



- QC Compare: Compares the spectrum with those in the specified “QC” libraries. Can change which libraries to search through the library manager button. Verifies quantitative composition of materials.



- Library Manager: Control which libraries to search in both the “QC” and general search options. New libraries can be added or created through the manager.