

PerkinElmer Spectrum 100 Infrared Spectrometer with ATR

Operating the Spectrometer and Software



LOCATION

Lee Science and Health Careers, Room 341

PROTECT THE CRYSTAL!

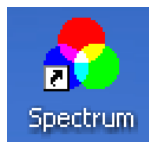
The attenuated total reflectance (ATR) accessory contains a diamond crystal, which is very hard and scratch resistant (knoop# = 7000 compared to 137 for Ze-Se). However, the crystal and the epoxy holding it in place can be damaged by excessive force and some chemicals.

1. Do not apply strong acids or bases.
2. Do not clean the crystal with acetone. Doing so may damage the crystal epoxy. Use absolute ethanol, isopropanol, methanol or dichloromethane.
3. Ensure solid samples are soft or smooth (e.g, piece of plastic bottle is fine). Grind crystalline solids in a mortar first. Hard, coarse solid may dislodge the crystal.
4. Do not apply pressure device directly to the crystal without a sample.
5. Do not drag pressure device across crystal. Turn counterclockwise first.

CLEAN THE CRYSTAL

Clean the crystal before and after acquiring sample data. You may remove organic residues with absolute ethanol, isopropanol, methanol or dichloromethane. To prevent spills and dripping, never apply solvent directly to crystal. To clean:

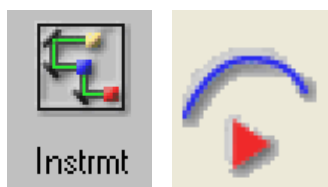
6. Dampen a Kimwipe, Q-tip or cotton ball with solvent (wear gloves; use a pipette or squirt bottle) and then wipe residues from diamond and pressure-tip. Repeat as necessary. Significant pressure or scrubbing may be required to remove solids from crystal.
7. Dispose cleaning materials into container next to IR.
8. Ensure solvent has sufficiently evaporated before acquiring background or sample.

SETUP

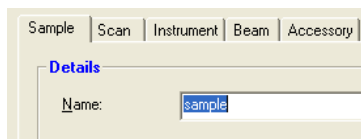
9. Ensure that the IR is on (it should always be on). Check LCD screen on instrument.
10. Login to the **PERKIN-IR** account on the PC (ask instructor for password).
11. Open the **SPECTRUM** software from the desktop icon.
12. When prompted for the **PERKINELMER LOGIN**, select **ANALYST**. No password is required.
13. When prompted, select **SPECTRUM 100** as the instrument and ensure **NO** is toggled for activate IR assistant.
14. Your instructor may direct you to select **OK** to any service or desiccant warnings.

ACQUIRE BACKGROUND - MANUAL

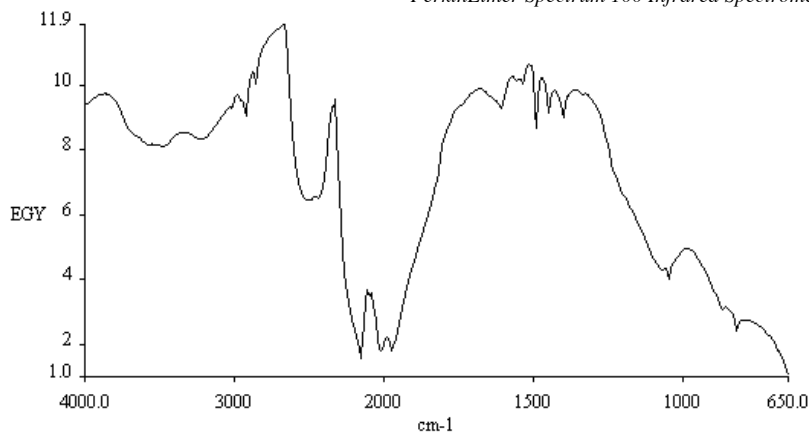
A background does not need to be collected before every sample acquisition, but it must be collected before the first sample acquisition after opening the software. You may collect a new background at any time.



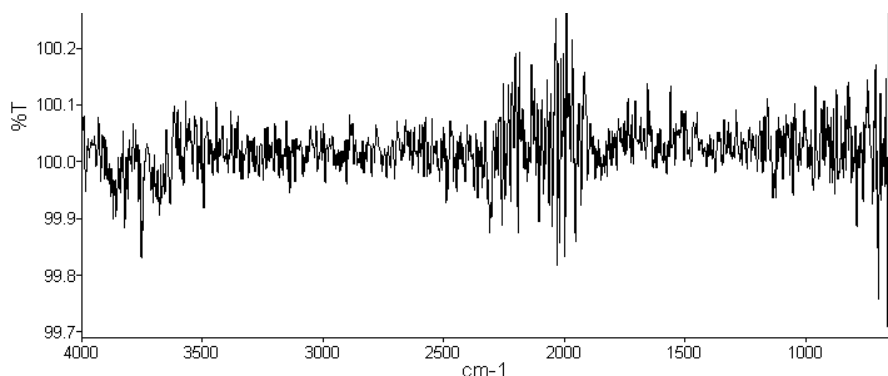
15. Ensure the crystal is clean and that the pressure device is not in contact with the crystal.
16. Click the **INSTMT** icon or go to **INSTRUMENT → SCAN**.
17. Click on the background icon (top right of screen; play button).
18. A new screen will open. Press **SCAN** when ready to collect background. You may view the scan progress in the lower left of the screen..

PREPARE TO ACQUIRE SAMPLE

19. In the scan and Instrument Setup window (select **INSTMT** icon to display if not already open), select the **SAMPLE** tab.
20. Enter a name for your sample in the box **NAME**. This will also be the filename. Be descriptive so that you can easily retrieve your data later if needed. A good example might be: *exp13-steamDist-kellyTomGrace*.
21. Ensure that **PREVIEW** is selected toward the bottom of the screen.
22. Your instructor may direct you to change parameters such as the number of scans in the **SCAN** tab. Generally, this is not necessary and the default values can be used.
23. Select **APPLY**.
24. Select **START**.
25. If a background was not previously acquired, a message at the top of the screen will read, **BACKGROUND REQUIRED**. Follow all direction and select **SCAN** to acquire background. A typical background with an ATR accessory is shown below.



26. If no background is required, a message at the top of the screen will read, **MONITORING. . . PLACE SAMPLE ON CRYSTAL.**
27. Before you apply your sample, observe the preview scan on the screen. It should appear to be noise across all wavelengths with close to 100% transmission since no sample has been placed on the crystal yet (see below). If significant bands appear, select **CLOSE**, clean the crystal and manually collect a new background.



APPLY AND ACQUIRE LIQUID SAMPLE

The pressure device is not needed for liquid samples. Liquids naturally make close contact with the crystal. Do not use the pressure device for liquid samples.

28. Add a small drop of liquid to the crystal with a pipette.
29. View the data in the **SCAN PROGRESS** screen. If the bands are weak, apply more sample. When the data is acceptable, press **SCAN** to collect the data.
30. Clean the crystal for the next user (see above) when finished.

OR APPLY AND ACQUIRE SOLID SAMPLE

31. Add a small portion (less than a spatula tip) of solid to the crystal with a spatula. The solid should be soft and powdered to prevent scratching the crystal.
32. Swing the pressure device over the crystal and turn the knob clockwise until the anvil makes contact with the solid.
33. Observe the Force Gauge on the screen. Continue turning the knob clockwise until the Force Gauge bar is green. When suitable bands are seen in the preview screen, stop turning. If the Force Gauge bar is red, excessive force

has been applied, which may damage the crystal. Turn the knob counterclockwise to reduce the force.

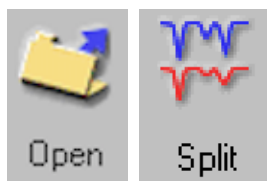
- View the data in the **SCAN PROGRESS – PREVIEW SAMPLE** screen. If the bands are weak either apply more force or unscrew the pressure device and add more sample. If the data is acceptable, press **SCAN** to collect the data.

SAVE SPECTRUM

- Before processing, save your spectrum. Click on the **SAVE ICON** or select **FILE → SAVE AS**. All spectral data have the extension *.sp. Peak picking and other processing changes will not be saved.
- Choose the folder where you wish to save your spectra. Your instructor should have a folder setup for your class within the **SPECTRA** folder (c:/pel_data/spectra). You may use the default filename, which is the sample name you entered previously or enter a new filename.

MULTIPLE SPECTRA

You may acquire additional spectra or open a saved spectrum (**FILE → OPEN**) and add to the same graph window. To overlay or split the spectra click on the **SPLIT ICON** until the desired view is observed or go to **VIEW → OVERLAY/SPLIT**. When multiple spectra are loaded into one graph window, each filename will be listed at the bottom of the screen with a color coded legend. You may need to expand this section to view all titles. To make changes to a single spectrum, select the title corresponding to that spectrum. To select more than one spectrum, hold **CTRL** down while clicking on the desired titles. By default all titles are selected when opening multiple files. Selected files are shown in bold.

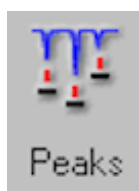


PEAK PICKING

Threshold Method:

Normally the default threshold settings are sufficient. These may be changed by your instructor if necessary (**SETUP → OPTIONS → PEAK TAB**).

- Select the desired spectrum/spectra from the legend at the bottom of the screen. Selected spectra will be bold. Hold **CTRL** down to select more than one spectrum. The following actions will be applied to all selected spectra.

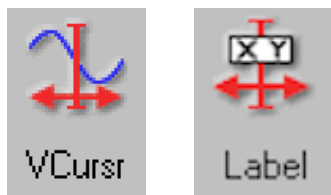


- Select the **PEAKS ICON** or Go to **VIEW → LABEL PEAKS**.

Individual Cursor Method:

This method may be used in conjunction with the threshold method to label missed peaks.

- Select the desired spectrum/spectra from the legend at the bottom of the screen. Selected spectra will be bold. Hold **CTRL** down to select more than one spectrum. The following actions will be applied to all selected spectra.



- Click the **VCURSR ICON** or go to **VIEW → CURSOR → VERTICAL – CONTINUOUS**.
- Drag the vertical cursor (green) to the peak you wish to label. You can view the x,y values at the bottom of the screen next to the legend. Enlarge this area if not seen.
- To apply label, select the **LABEL ICON** or go to **VIEW → LABEL CURSOR** or double click on the green line.

ADD TEXT ANNOTATIONS

- Add titles/label each spectrum. Click on the **TEXT ICON** or go to **VIEW →**



ADD/EDIT TEXT.

44. Type in an appropriate title and select **OK**.
45. Drag text to desired location in the graph.
46. Repeat as needed.

SAVE GRAPH – OPTIONAL

47. You should have already saved your spectrum data in the steps above. You may also save your graph window, which includes all multiple spectra, peak picking and text annotations. To do so, select **FILE → SAVE AS** (Do not select **SAVE!**).
48. Enter a filename. You may use the same filename as before.
49. Choose **GRAPHS (*.GPH)** for **SAVE AS TYPE**.
50. Choose a folder. Your instructor should have a folder setup for your class within the **SPECTRA** folder (c:/pel_data/spectra).
51. Select **SAVE**.
52. Note: If you'd like to open a graph, be sure to choose **GRAPHS (*.GPH)** for **FILES OF TYPE**.

PRINTING

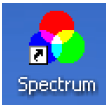
53. Select the **PRINT ICON** to print using the most recently used settings.
54. Or go to **FILE → PRINT**.
55. Choose the correct **PRINTER** if not already listed.
56. For **OUTPUT** select **WITH REPORT TEMPLATE** if not already selected and choose the file, C:\pel_data\template\landrieOrgoClasses.pfm, if not already loaded.

With No Titles or Formatting:

57. For **OUTPUT** select **CURRENT WINDOW**.



Quick Start Guide



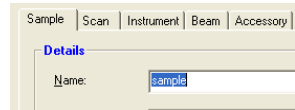
1. Open **SPECTRUM** software.



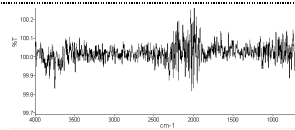
2. Open **SCAN AND INSTRUMENT SETUP** screen.



3. Clean crystal, then manually acquire **BACKGROUND** (optional).



4. Select **SAMPLE TAB**. Enter **SAMPLE NAME**. Click **APPLY**. Click **START** to begin preview scans.



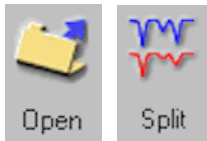
5. Observe noise (no bands).

6. Apply sample. Apply force for solids.

7. Select **SCAN** to acquire data.



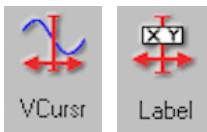
8. Save spectrum in c:/pel_data/spectra folder or subfolder.



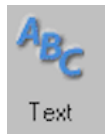
9. Acquire additional spectra or **OPEN** additional spectra. **OVERLAY** or **SPLIT** spectra in graph window.



10. Peak Picking: Label all peaks.



11. Label individual peaks with **VERTICAL CURSOR**.



12. Add text annotations such as titles.

13. Save as graph. Choose **GRAPHS(*.GPH)** from **SAVE AS TYPE**. (optional)



14. Print.

What is Attenuated Total Reflectance Anyway?

The attenuated total reflectance (ATR) accessory allows us to acquire spectra in a matter of seconds. The reason? There is little sample preparation required. Just apply the sample to the crystal surface, apply pressure if it's a solid, start scanning, and voila. Compare this to transmission infrared (IR) spectroscopy. Here the sample must be transparent since the infrared beam will be passing through it. That's usually not a problem in the case of liquids. For solids, however, you would either need to prepare a solution or use a number of more complex techniques such as dispersing the solid in KBr pellets or Nujol mulls. As light passes through the sample, specific frequencies of infrared light are absorbed corresponding to the frequency of molecular bond movements such as stretching and bending.

In ATR, the IR beam does not pass through the sample, but rather is reflected off the sample (Figure A5.2) that is applied to a high refractive index crystal. As long as the refractive index of the crystal is greater than that of the sample, and the IR beam enters at an angle greater than the critical angle, total internal reflectance occurs. As the light bounces off the inside of the crystal surface, a small amount of radiation penetrates the sample and then returns to the reflected IR beam. Since the intensity of this light decays exponentially with distance (an evanescent wave) it can only penetrate the sample a few micrometers (μm). (For comparison, the diameter of a human hair is 17-180 micrometers.) This is the reason solids must be compressed onto the crystal surface, to ensure there is no air gap between the crystal and solid.

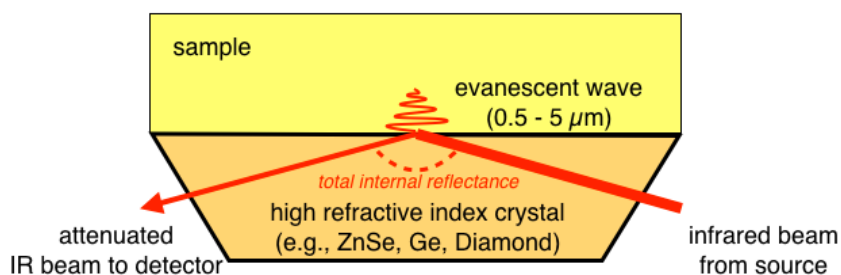


Figure A5.2: Evanescent wave upon total internal reflection of infrared beam.

The energy of the evanescent wave is attenuated (magnitude decreased) by absorption of the characteristic vibrational frequencies of the sample. The attenuated wave then returns to the path of the IR beam and continues to the detector.

COMPARISON OF ATR AND TRANSMISSION SPECTRA

Despite the drastically reduced sampling time, ATR spectroscopy does present some challenges. The spectrum obtained from ATR spectroscopy is not identical to a spectrum obtained through transmission techniques. ATR causes reduced band intensities as well as small, but significant shifts in absolute frequency toward lower wavenumbers. If an ATR spectrum representative of a transmission spectrum is desired (e.g., for comparing to spectral libraries) the ATR spectrum should be *corrected*. After correction, the peak positions and intensities more closely resemble those of transmission data. However, a corrected ATR spectrum is still only an approximation of the data that would have been collected in transmission mode. It's important, therefore, to always list the method of acquisition when reporting IR data.