Advantage Series User Manual
NuSpec Software
532 – 633 – 785

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CE Declaration of Conformity

Manufacturer: DeltaNu Inc.
5452 Old Highway 130, Laramie, WY 82070
307.745.9148, Fax 307.745.9152
www.deltanu.com, sales@deltanu.com

Model: Advantage Systems

Type of Equipment: Raman Spectrometers

Testing Provider
and Certification by: Communication Certification Laboratory
Salt Lake City, Utah

Tests: Emissions:
EN 55011: 1998, FCC Part 15, Subpart B, ICES-003
Test Serial No: 73-8004

Immunity:
EN 55024: 1998, EN 61000-3-2, EN 61000-3-3
Test Serial No: 83-0972

Safety:
EN 61010-1:1990 + A1:1992 + A2:1995
Test Serial No: 89-0667

I, the undersigned, hereby declare that the equipment above conforms to the above Directives and Standards, when used in accordance with the manufacturer's specifications.

Place of Issue: Laramie, WY

Date of Issue: October 20, 2004   Keith Carron, General Manager

1.0 Introduction

Congratulations on your purchase. The Advantage series spectrometers are easy to use on solutions, gels, powders, and coatings. The Advantage series covers a wide spectral range for analysis of inorganic anions to aliphatic hydrocarbons. It is used in industrial applications, academic research, and teaching labs.

This manual will lead you through initial set-up, steps for taking a spectrum, and a thorough description of the DeltaNu software and features. Other sections in this manual will help you improve spectra by describing the product software features in more detail, provide basic troubleshooting for the most common problems seen while operating, and contact information for problems not found in the manual. Also included are pictures and descriptions of all attachments available for use with the Advantage series spectrometers, along with guides for installation.

1.1 Unpacking and Checklist

Using the checklist found in the shipping container, or the following list, review and make sure all parts are accounted for.

The following items come standard with an Advantage series spectrometer.
- □ Advantage series Raman Spectrometer
- □ Sample Holder Assembly
- □ Melting Point Tube Adapter
- □ NMR Tube Adapter
- □ Vials (250 8mm sample)
- □ Jump Drive or CD
  - o DeltaNu Software
  - o SPC Viewer
- □ Instruction Manual
- □ Power Supply
- □ Power Cord
- □ USB Cable
- □ 1/8" Hex T-Handle Wrench
- □ 3/16" Allen Wrench
- □ Extra Desiccants
- □ Laptop PC with Kensington Lock
- □ Library Management Software

The following items are accessories for the Advantage series spectrometer.
- □ XYZ Stage
  - o Magnetic Interlock Override
  - o 9/64" Hex Wrench
- □ Input Optics Extension
- □ Right Angle Input Optics
- □ NuScope
  - o 5/32" Allen Wrench
  - o 5/64" Allen Wrench

*Please note section 13.0; Changing the Desiccant. It is important to the overall performance of the instrument that the desiccant is checked every 3-6 months.
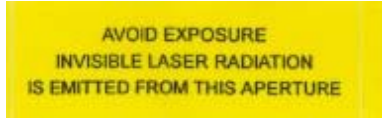
1.2 Laser Safety Warnings

Fig. 1 (Laser safety label)

Aperture warning for Advantage 532
When acquiring data the system will emit up to 100mW at 532 nm of radiation through its output optics.

Aperture warning for Advantage 633
When acquiring data the system will emit up to 3mW at 633 nm of radiation through its output optics.

Aperture warning for Advantage 785
When acquiring data the system will emit up to 60mW at 785 nm of radiation through its output optics. This hazard is specified by the Nominal Ocular Hazard Distance (NOHD). **The Advantage NIR has an NOHD of 36 inches (91.4 cm).** This represents the distance at which the radiation has decreased to $2mW/cm^2$.

Caution **– use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure. Only trained, authorized personnel should be allowed to use this instrument. Occasional maintenance may be required to keep the output optics clean. All maintenance should be performed with the system off.**

Fig. 2 (Laser Warning Label)

2.0 Diagram of Advantage system

Looking at the back of an Advantage series spectrometer, in the top left corner is the *ON/OFF* switch and the serial number plate. Further to the right, locate the 12 volt DC connector and the USB connection.

Fig. 3 (Back of Advantage Series Spectrometer)

On the front of an Advantage series spectrometer there are two small LED's. On the left, a laser cooling light, and on the right, a laser *ON* light.

Fig. 4 (Front of Advantage NIR Spectrometer)

3.0 Taking a Raman Spectrum

The Advantage series spectrometers are specifically designed for ease of use. Spectra of liquids or solids in standard sample vials are obtained by placing the vial in the sample holder. It is recommended that the sample vial contain approximately ½" sample material to ensure detection by the laser beam in the first few weeks of use. As the instrument becomes more familiar to the user, it is possible to use very small amounts of sample material. Simply adjust the sample vial vertically within the sample holder to ensure alignment of the sample and the laser beam. Cyclohexane or toluene provides an excellent liquid sample to practice adjusting the sample holder. Acetaminophen (Tylenol) or benzoic acid are good solid materials to use for practice in focusing.

Once the sample is in place, ensure that the integration time is on 1 second and press the *Continuous* button. The system will acquire a reference spectrum (with a shutter blocking the laser beam) and then continually acquire spectra (with the reference subtracted out) every second. This real-time mode allows the user to observe changes in the spectrum while manipulating the sample holder.

Now that the system is displaying real-time spectra, properly focus the laser beam onto the sample material by adjusting the silver knob on the front of the sample holder. For liquids the focus is not critical, and there is a large range over which the adjustment makes very little change in the spectrum. When the sample is far away from the instrument, there is a decrease in signal and perhaps an increase in background as the glass sample vial itself moves into focus. Similarly, if the sample is too close, the sample vial will dominate the spectrum. Once the laser is focused for optimal signal, the user should not have to make adjustments. However, the distance is dependent on the index of refraction of the sample and may differ slightly with different samples. Solids are optimized the same way. If the solid is opaque, the optimization is more critical than with liquids. The ideal distance will be that at which the laser is focused just on the sample material, and not the glass vial. The Advantage series have an interlock that shuts the laser off if the sample holder is removed. If the laser turns off while focusing because the interlock has been tripped, focus back and restart the software.

Once the intensity is optimized and the background noise is minimized, click the *Continuous* button again to stop the acquisition. Now, adjust the integration time to the desired time and click *Acquire*. The integration time required to obtain a spectrum depends on the sample and the application.

The Advantage series detector begins to saturate around a signal greater than 65,535 counts per second. To obtain the best signal to noise ratio, keep the largest peak in the spectrum close to, but under, 65,500 counts per second.

4.0 NuSpec Software

A figure showing the software user interface window is illustrated below (**Fig. 5**) with a further explanation of all its features. *A DeltaNu system must be on and connected for the software to work properly.*

4.1 Spectrometer-Specific User Interface

Certain controls do not apply to some spectrometers. For example, not all spectrometers have the *Polarization* feature or automatic integration time. When you connect to a spectrometer, the software user interface hides the controls that do not apply to that spectrometer.
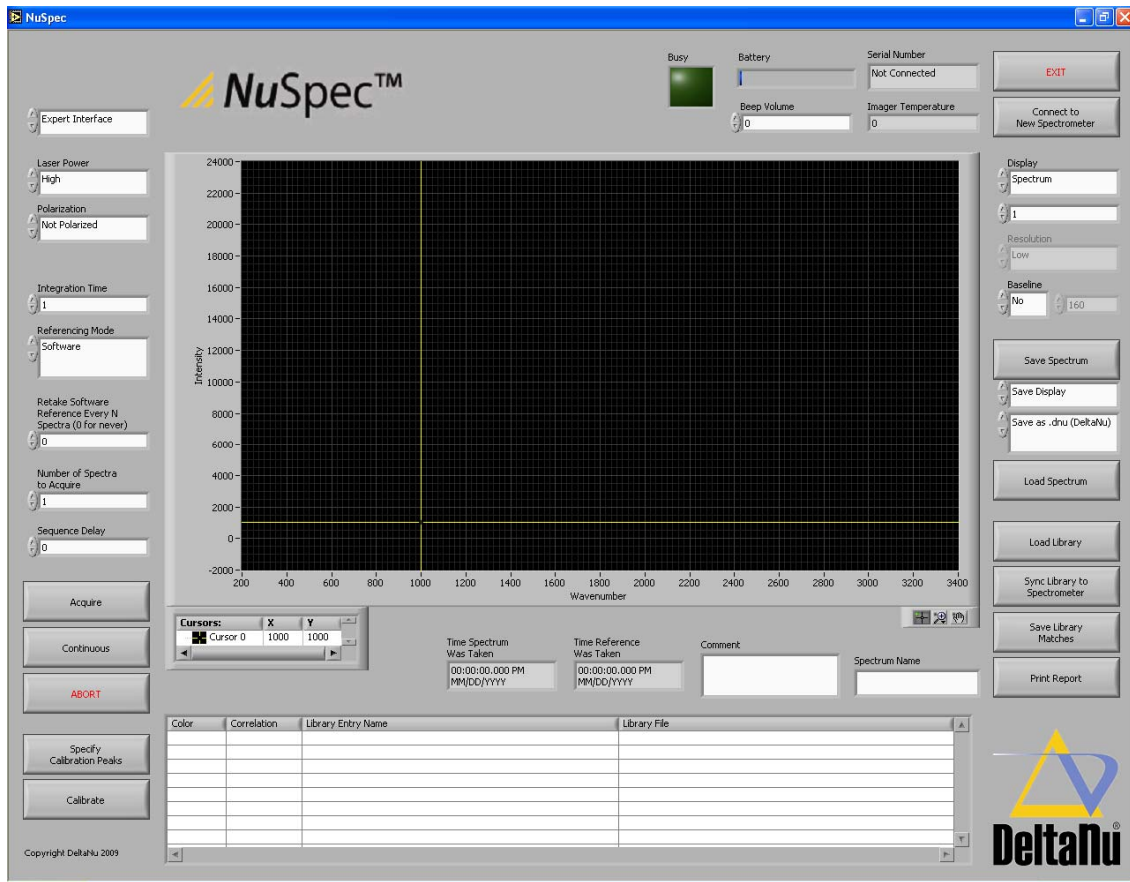

Fig. 5 (NuSpec software screen)

Select the NuSpec software from *C:\Program Files\DeltaNu* or from a Desktop shortcut that you may have created. When the software is first opened, the computer port that the spectrometer is plugged into must be selected before continuing (**Fig. 6**). This tells the software where to locate the spectrometer. If the correct port number is not initially displayed in the pull-down menu, then follow the instructions that are given on the screen to add the correct port to the list so that it can be selected.
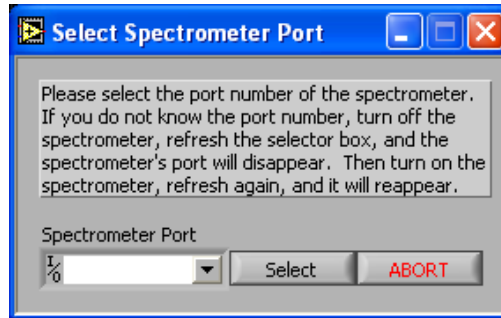

Fig. 6 (Select spectrometer port)

The first time the spectrometer is connected to a computer the *Create Spectrometer Parameter File* window will display (**Fig. 7**). Follow the onscreen instructions to continue. This window is used to specify the range of wavenumbers for the system, the imager temperature, and the firmware version. By simply clicking on the button that corresponds to your system (i.e. ExamineR 532) the default parameters of that system will be automatically loaded on this screen. *Note: This step is only required the first time the spectrometer is connected to the computer. This window will not appear on subsequent connections.*

Fig. 7 (Create spectrometer parameter file)

4.2 Disconnected Mode

When the software prompts you to select the spectrometer computer port, if you click ABORT the software will go into the "disconnected" mode. It will also go into disconnected mode if there is an error while attempting to connect to a spectrometer. In this mode, the serial number display will read "Not Connected". While you are "Not Connected" you can still upload spectrum files and library files, view the spectra, and perform sample matching. Any of the controls on the left that communicate with the spectrometer directly will be ignored. This can be a useful mode to do off-line data analysis.

*Note: If you connect to a spectrometer and its clock is set different than the computer's clock it will ask you if you want to synch the spectrometer's clock to the computer (**Fig. 8**). Click OK to do so.*
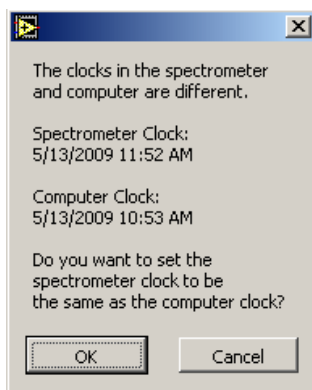

Fig. 8 (Synch spectrometer clock)

4.3 Serial Number

The spectrometer is properly communicating with the software if the serial number of the device is displayed in the *Serial Number* box found in the upper right hand corner of the viewing window (**Fig. 9**). The software will automatically display the serial number in the box if the spectrometer is properly connected, turned ON, and the correct computer port was selected. The user can select the *Connect to New Spectrometer* option to use another DeltaNu system while the software is running.
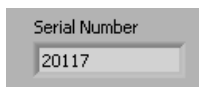

Fig. 9 (Serial Number)

4.4 System Calibration

Each user should initially setup a calibration parameter file with the software. Such parameters are used to correlate the pixels of the spectrum viewing window to a specific range of wavenumbers, and to standardize the device to the polystyrene ASTM standard. This feature allows the software to determine the peak windows in which to search for the polystyrene ASTM file. It is recommended that you select "Automatic" for the *Integration Time* in order to get the best calibration. Calibrating the system is only required if you have a lower correlation match than 0.95 between your polystyrene standard and the polystyrene calibration file stored in the library.

4.4.1 Specify Calibration Peaks

This is the first step in calibrating your polystyrene ASTM standard. After you have placed a vial of the calibration standard in the vial holder, select the *Specify Calibration Peaks* button on the lower left hand side of the software screen. You will be redirected to a new screen and a spectrum of your standard will be displayed. Four polystyrene peaks are pre-selected and shown in the table and figure below (**Fig. 10**). The highlighted peak values will be used in this calibration step.

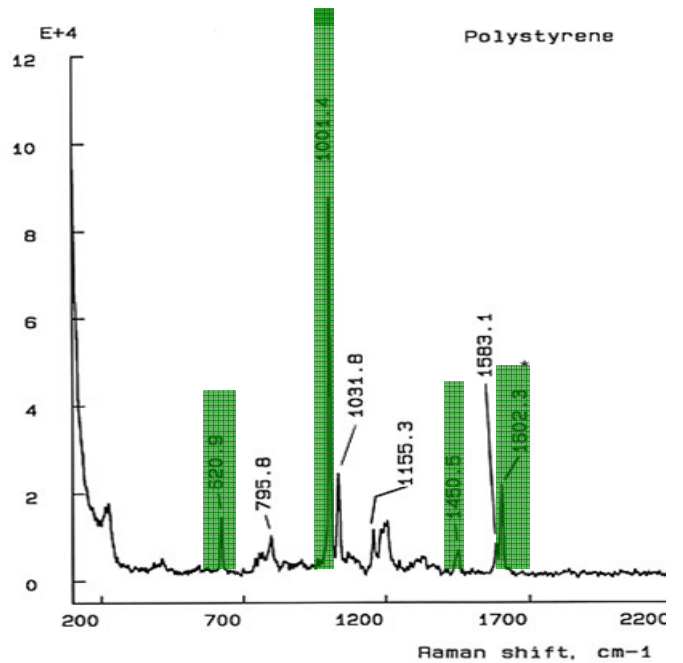| Sample	Mean Frequency	Standard Deviation	Number of Points	Relative Intensity
Polystyrene	620.9	0.69	6	16
	795.8	0.78	6	10
	1001.4	0.54	6	100
	1031.8	0.43	6	27
	1155.3	0.56	6	13
	1450.5	0.56	6	8
	1583.1	0.86	6	12
	1602.3	0.73	6	28
	2852.4	0.89	5	9
	2904.5	1.22	5	13
	3054.3	1.36	6	32

Fig. 10 (Calibration Peaks)

There are several colored bars on the calibration parameter screen (**Fig. 11**). Each color corresponds to a particular wavenumber (i.e. red = 620.9). Move the colored bars over the corresponding peaks as needed to match the wavenumbers with the correct peaks. The colored bars can be selected and moved on the window by left-clicking the mouse on the bars and dragging them over the correct peaks. After you have correctly identified the peaks, you can click the *Save* button to keep that calibration file, or click *Retake* to obtain another calibration spectrum.
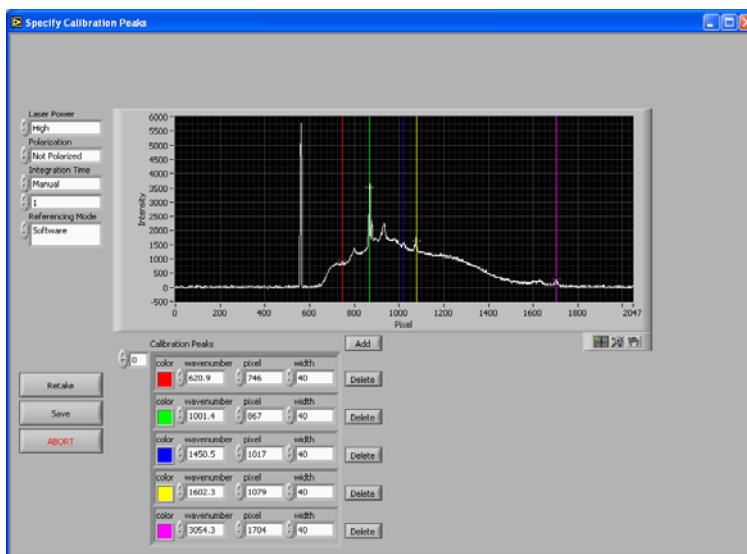

Fig. 11 (Calibration parameters)

4.4.2 Calibrate

After you have saved your calibration peaks on the previous window you will now be redirected back to the software's main viewing window (**Fig. 5**). Now you are ready to calibrate the system to your polystyrene ASTM standard. With the calibration standard still in the vial holder, click on the *Calibrate* button. When the calibration is complete a window will open with three selections (**Fig. 12**): "*Keep new calibration*", "*Keep old calibration*", and "*Retry calibration*" along with an *rms error* value. The lower the *rms error* value the better the calibration. If you choose to save this *rms error* number for your calibration it will be saved in the *default.sdb* file. This file is not readable, but can be validated by the date stamp. Re-calibrate if the *rms error* value is greater than 2.0.
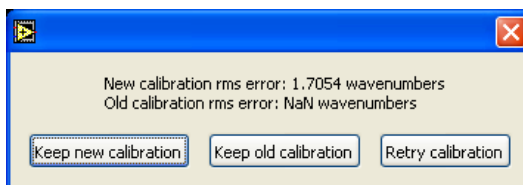

Fig. 12 (New calibration)

4.5 Basic vs. Expert User Interface

Selecting "Basic Interface" allows the software to automatically use default settings for many of the basic controls. These control buttons will be hidden on the viewing window (**Fig. 13**). This provides an easier-to-learn interface for casual users. Selecting the "Expert Interface" gives access to all of the controls that are available for the current spectrometer.
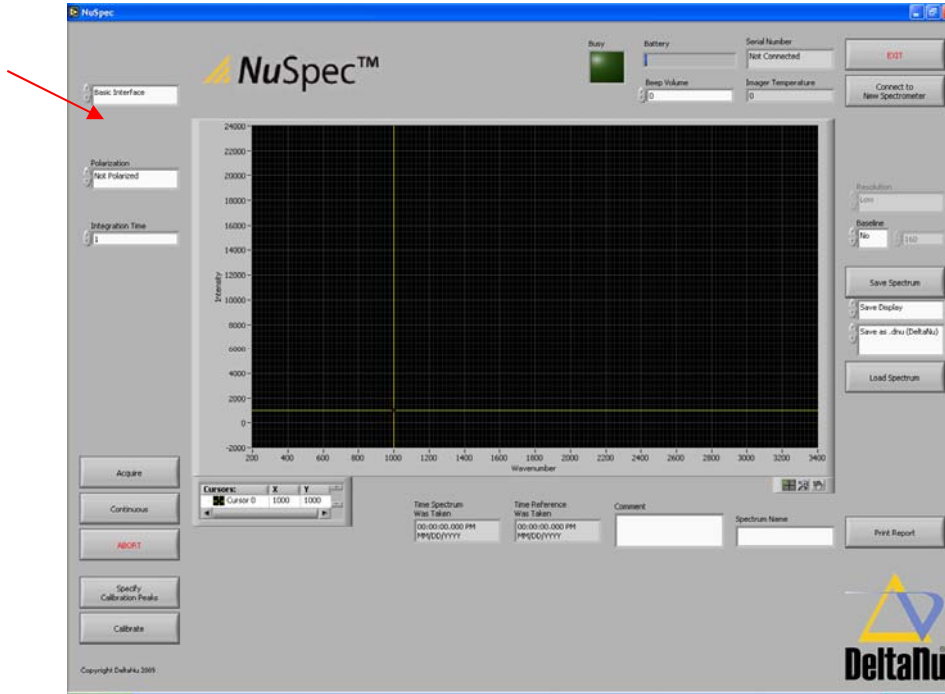

Fig. 13 (Basic user interface)

4.6 Laser Power

The *Laser Power* bar selects the power of the laser from five settings ranging from low to high (**Fig. 14**). It is normally best to use the "High" power setting for the laser. This will provide the best signal to block out background noise. However, some samples may be burned or destroyed under "High" laser illumination. When sample degradation is observed a lower power setting is suggested.
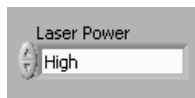

Fig. 14 (Laser power)

4.7 Polarization

This feature automatically subtracts molecule polarizations from the spectrum in order to provide information about the molecular symmetry. *The polarization feature can only be found on Advantage 532, Advantage 633, and Advantage 785 software.*

Raman spectra contain special information about the polarization of the scattered light. Bands that are highly polarized correspond to totally symmetric modes. This can be used to aid in the characterization of Raman features in a spectrum.
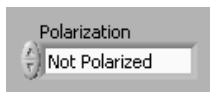

Fig. 15 (Polarization)

4.8 Integration time

The *Integration Time* feature has two modes (**Fig. 16**): Automatic and Manual. Selecting the automatic feature means the integration time utilizes the same acquisition parameters as the device hardware. The maximum default setting for the hardware is 60s. In this mode, the device selects the optimum exposure time based on the collection signal obtained in a 0.3s check of the sample. It is recommended that library entries are saved using this automatic mode.

The manual feature allows the integration time to be changed. In the manual mode the integration time cannot be set higher than 60 seconds. The lowest setting is 0.01 seconds. The integration mode and time can be changed using the arrow keys to select an option.
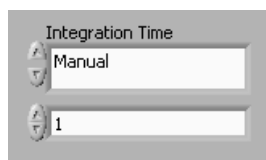

Fig. 16 (Integration time)

4.9 Referencing Mode

"Software" referencing mode requires the manual *Integration Time* setting. Automatic *Integration Time* cannot be used with "Software" referencing so that option is hidden when "Software" referencing mode is selected. To use automatic *Integration Time* select either "Hardware" referencing or select "None". To take a new reference select "retake software reference only" and click acquire. The reference spectrum will be displayed on

the graph and you can save it like a normal spectrum if you want to use it for further analysis. A new reference spectrum is taken every time the integration time is changed. Users should also take a reference when the ambient light has changed.
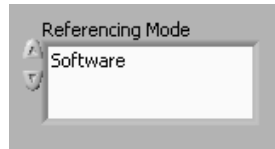

Fig. 17 (Referencing mode)

4.10 Number of Spectra to Acquire

This feature is used to acquire a specified number of spectrum consecutively that can then be "averaged" together. Select the number of spectrum to acquire using the up and down arrows or the number pad on the keyboard (**Fig. 18**). Click the *Acquire* button to collect the spectra. To view the "averaged" spectrum or the individual spectra use the *Display* feature to select the option of choice (see *Display* section below).
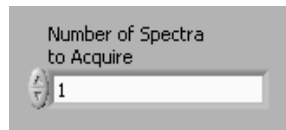

Fig. 18 (Number of spectra to acquire)

4.11 Display

The *Display* feature allows the user to switch between showing the individual spectrum acquired or the "averaged" spectrum. To view a specific spectrum in the averaged series select the display "Spectrum" option in the top box (**Fig. 19**) and use the arrows on the box below to scroll through the spectra numbers. To view the averaged spectrum simply select the "Average" display option in the top box.
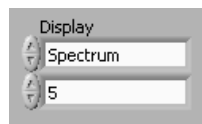

Fig. 19 (Display)

4.12 Sequence Delay

The *Sequence Delay* feature can be used when acquiring multiple spectra at once to insert a specified time delay in between acquiring each spectrum. The time is set in seconds from the start of the first acquire to the start of the next acquire. If the delay is less than the specified integration time then each spectrum is acquired for the full integration time and the next acquire starts as soon as the previous one is done.
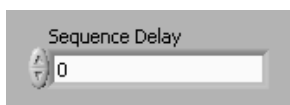

Fig. 20 (Sequence delay)

4.13 Acquire

Clicking on the *Acquire* button turns the laser on and begins the acquisition of the spectrum. At this point, the software will collect as many spectra consecutively as you have specified.

Note: When more than one spectrum is acquired it can be saved as a multi-file or as a single "averaged" file. The procedure to save a single-file or a multi-file is further described in the section below titled "*Save Spectrum*".

Fig. 21 (Acquire)

4.14 Busy

The *Busy* light (**Fig. 22**) in the top right corner of the viewing window is on when the software is busy. Note: This light is not an indication of the laser being ON or OFF.   For example, when an *Acquire* action is performed, the *Laser Warning* indicator will turn off when the laser goes off, but the *Busy* light will stay on while the software takes a reference.

Fig. 22 (Busy light)

4.15 Laser Warning

The laser warning symbol is shown next to the busy light only when the laser is ON.

Fig. 23 (Laser warning)

4.16 Continuous

Clicking the *Continuous* button allows the laser to run continuously until stopped. This mode is used to focus or position the laser on the sample with the optimum signal collection. To stop the continuous mode, click the *Continuous* button again.
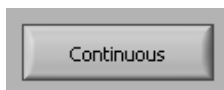

Fig. 24 (Continuous)

4.17 Abort

The ABORT button will cancel a reference, an acquire, or a continuous function when clicked.

Fig. 25 (Abort)

4.18 Resolution

The *Resolution* setting can be changed from Low, Medium-Low, Medium, Medium-High, or High using the pushbuttons on the left of the box. The benefit of using a low resolution setting is that you get less background noise. However, high resolution allows one to see spectral features that would not be separated at lower resolution. *Note: It is recommended that all library spectra be saved at a low resolution setting.*
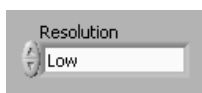

Fig. 26 (Resolution)

4.19 Baseline

The *Baseline* feature can be turned ON or OFF ("Yes" or "No") using the pushbuttons on the left. When the baseline feature is ON the fluorescence is subtracted from the spectra giving a "smoothing" effect to the spectra. The number allows the user to fine tune the baseline algorithm to account for differences in the width of peaks for different spectra.

Fig. 27 (Baseline)

5.0 Saving Spectrum

The *Save Spectrum* button allows the user to save spectra to specific library folders. The spectrum can be saved as displayed on the screen (Save Display) or as raw data (Save Raw Data) by using the arrow keys next to the top box (**Fig. 28**).   Next, you can choose what type of file to save it as using the arrow keys on the second box. Files can be saved as .dnu, .spc, or .prn extensions. Once you have chosen the file type click on the *Save Spectrum* button, choose a library or folder in which to save the spectra file, and give the file a name. *Note: To save a library file it must be saved with the "Save Display" function.*
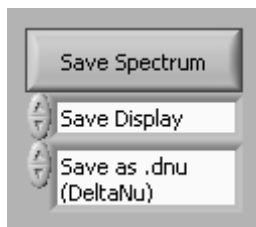

Fig. 28 (Save spectrum menu)

5.1 File Types

Library entries used for the library builder may be saved as .spc, .dnu, or .prn files. However, it is recommended that library entries are saved as .spc files. If you wish to be able to load a spectrum back into the NuSpec software, then save it as a .dnu file to preserve all of the metadata. To be able to load a spectrum into GRAMS save it as a .spc file. To load it into Microsoft Excel it must be saved as a .prn file.

5.2 Saving Averaged Spectra

An "averaged" spectrum can be obtained to minimize the noise in the spectra. Typically, no more than 10 acquired spectra are needed to minimize noise. An average file is sometimes used as a library entry. First, select the number of spectra to be "averaged" by using the arrow keys next to the *Number of Spectra to Acquire* function and then click on the *Acquire* button.

Note: You can use the *Retake Software Reference Every N Spectra* function to force it to retake a reference for every "*N*" number of spectra acquired by choosing a value for "*N*".

1) After the analysis is complete, select "Average" in the *Display* window using the arrow keys on the top box (**Fig. 19**).
2) Select the desired resolution level (low, medium, or high). If "*Save Raw Data*" is selected the spectra is automatically saved at high resolution. If "*Save Display*" is selected it saves at whatever resolution setting is currently chosen. *We recommend that library entries be saved at low resolution.*
3) Select "Save Display" using the arrow keys under the *Save Spectrum* button (**Fig. 28**). Save the averaged spectra in a folder or as a single library entry.

5.3 Saving a Multi-file

A multi-file is a single file that contains more than one spectrum. A multi-file is not commonly used for anything other than repeatability tests. It is NOT used for a library entry. It can only be saved using the "Save Raw Data" selection (**Fig. 28**). Another reason to save a multi-file is to load the spectrum back into the NuSpec software and manipulate resolution, etc. If the file is saved at low resolution the "smoothing" effect is applied permanently and cannot be undone. The integration time cannot be set higher than 10 seconds. The lowest setting is 0.01 seconds.

5.4 Load Spectrum

This feature loads a spectrum (.spc or .dnu file). A new window will open when you click this button (**Fig. 29**). Select the spectrum that you wish to load from the appropriate folder.
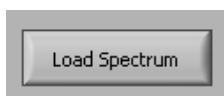

Fig. 29 (Load Spectrum)

5.5 Load Library

The *Load Library* feature loads a library of spectra from a specific folder. Clicking on the *Load Library* button will open a new window that allows library or spectra files to be added, selected or de-selected, or deleted. Click on the *Save* button to continue after your final selections have been made. To delete a spectra or library, click on the file name to highlight it and click on the *Delete* button on the bottom of the screen. To delete all the spectra/libraries listed click on *Delete All*. To exit this screen without making changes click on the *ABORT* button.
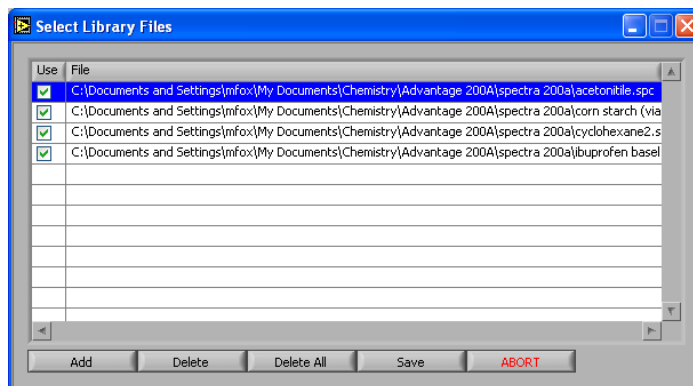

Fig. 30 (Load library)

The spectra or libraries that you selected will now be displayed on the main software screen (**Fig. 31**). To view the actual spectra click on the Library Entry Name at the bottom of the screen and the spectra will be displayed in the corresponding color of the highlighted entry name. To de-select the spectrum click on the entry name again. It is also possible to view multiple spectra at once by clicking on multiple entry names. Each spectrum will be displayed in a different color.

The library spectrum list can be sorted by correlation match or library entry name by clicking on the corresponding title bars at the top of the spectrum list (i.e. "Correlation" or "Library Entry Name").

The comment window allows further description of a sample/spectrum to be added and saved with the spectrum. The user can type in both the "Comment" and "Spectrum Name" fields and the changed values will be saved with the spectrum.
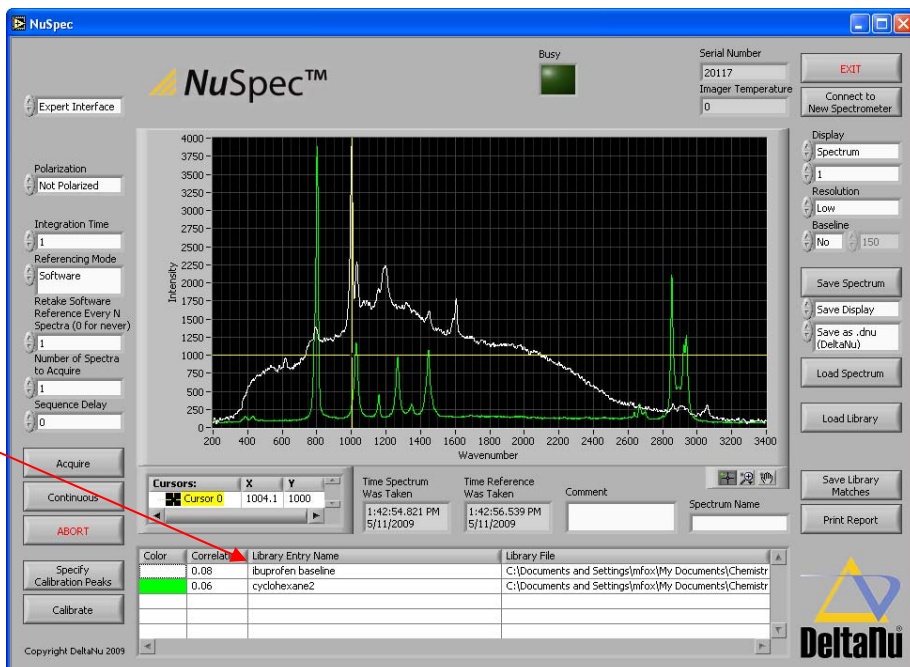
Fig. 31 (Library entry)

5.6 Sync Library to Spectrometer

The Sync Library to spectrometer button loads the currently selected library to the system. *For the ReporteR model only.*
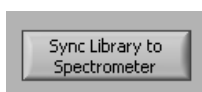

Fig. 32 (Sync library to spectrometer)

5.7 Display Screen

There are several features that allow the user to navigate the cursor on the spectrum viewing window, zoom in on specific peaks, and move the location of the viewing window.

The exact location of the cursor is numerically displayed (X and Y axis coordinates) below the spectra screen. Clicking on the cursor icon in this box allows the user to manually enter X and Y coordinates for the cursor.

The hand icon (**Fig. 33**) on the lower right hand side of the viewing window allows the entire window to be "pulled" to a specific point. After selecting the hand icon button,

left-click the mouse cursor on the spectrum screen and drag the entire window (without de-pressing the mouse button) where you want it.

Fig. 33 (Hand icon)

The magnifying glass icon (**Fig. 34**) allows the user to zoom in on the entire spectrum, specific areas of the spectrum, or specific peaks of interest. A window with different options will open when the magnifying glass button is selected.
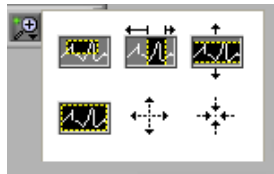

Fig. 34 (Magnifying glass options)

The cross icon (**Fig. 35**) allows the cursor to be moved to a specific location on the spectrum screen by clicking and dragging the cursor axis lines.
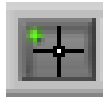

Fig. 35 (Cross icon)

The mouse can also be used to move the cursor on the screen. While the cross icon is selected, you can double click the left mouse button on the spectrum screen to center the screen on that point. You can also click the middle mouse button (or Alt+left button) on the screen to center the screen on the entire spectrum. Clicking Ctrl+left mouse button switches back and forth between the magnifying glass tool and the cross tool.

5.8 Print report

The *Print Report* function will print a screen shot of the NuSpec software. Select the printer and number of copies to be printed and select okay. To close this feature select ABORT.
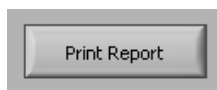

Fig. 36 (Print report)

5.9 Imager temperature

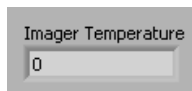The imager temperature displays the temperature of the CCD. This is updated every 30 seconds.

Fig. 37 (Imager temperature)

5.10 Exit

To close or exit the NuSpec software click on the *EXIT* button in the top right corner.

Fig. 38 (Exit)

6.0 Fluorescence

Challenges come when the sample has an inherent background due to fluorescence. In this case, background will also increase with the integration time and smaller improvements are gained by integrating longer. The Advantage shows no improvement by integrating longer than 60 seconds (the software does not allow integration longer than 59 seconds). If the sample is fluorescing it may saturate the detector long before the 60-second integration period. The best way to improve a spectrum in this case is to average over several spectra.

NuSpec offers a very unique feature that can also help with difficult samples. This is the *Baseline* feature. This feature performs a real-time baseline correction to remove spurious features such as fluorescence from the spectrum.

If the sample is a poor Raman scatterer or has a fluorescence background, the following procedure is suggested. Select *yes* under the baseline menu option and then click on *continuous*. Carefully adjust the sample position. In many cases, it is possible to find a position that maximizes the Raman signal, while minimizing fluorescence. The baseline feature is very useful as it often allows for observation of a Raman feature that would normally be hidden in a more spurious baseline. Once the position has been optimized, select an integration time that produces a good signal-to-noise ratio and, if needed, average over several spectra.

7.0 Resolution

The *Resolution* feature can be used to improve spectra. As a rule of thumb, in any dispersive spectroscopy (spectroscopy where a grating or prism spreads the spectrum across the exit plane of the spectrograph) the signal-to-noise is inversely proportional to the resolution. The Advantage series allows the user to select three resolution levels to improve signal-to-noise ratio. At low resolution the signal-to-noise ratio is optimal, but the spectral resolution is lower. This can be readily observed with a sample such as toluene. At the low-resolution setting the two large peaks around 1000 cm^{-1} are not resolved. However, note the excellent signal-to-noise ratio even at one second. As the resolution is improved, the two peaks are resolved at the cost of adding noise to the background. As a rule of thumb, many samples do not require high resolution and the best spectra are obtained in the low-resolution mode.

8.0 Ambient Light

We recommend that you characterize the ambient light conditions in the environment that you are working in. This test will provide you peaks that could possibly interfere when a poor reference is obtained. As an example, we have provided a spectrum that was obtained by referencing with a shroud or black optical cloth covering the sample vial attachment. After a reference was taken, a fluorescent light (turned on) was placed in front of the attachment and a sample of the light was obtained (**Figure 39**).
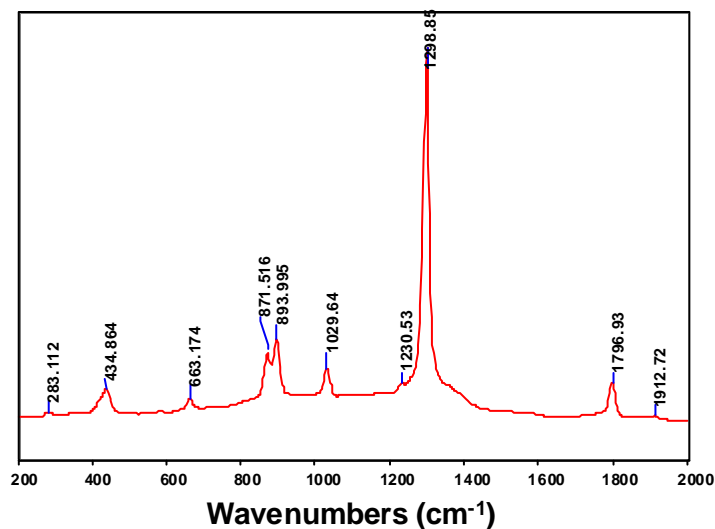

Fig. 39 (Ambient Light Example 785nm)

9.0 Glass Interference

Another issue that may arise when acquiring spectra is glass interference. For example, the manual adjustments on the focusing knob for the sample vial attachment allow minimal glass interference; however, certain experimental conditions may cause glass interference. One common mistake is not filling the vial full enough with sample. Consequently, Raman is collected on the glass above the sample. Interference due to fluorescence impurities in the glass may be observed near 1400 cm^{-1} **(Figure 40)** for the 8mm glass HPLC vials. This spectrum may vary based upon the type of glass material used.
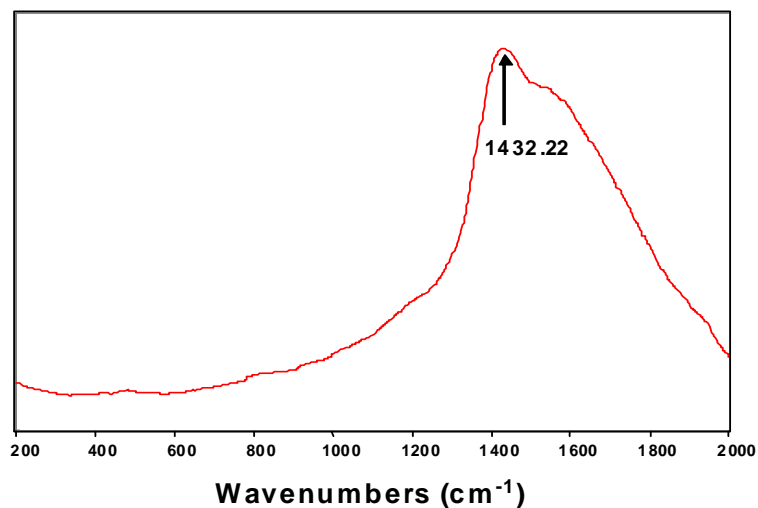

Fig. 40 (Glass Vial Example 785nm)

Melting point tubes and NMR tubes may have different spectral impurities, and acquiring a Raman spectrum of the sample container before each experiment is recommended. Figure 41 illustrates glass fluorescence while sampling through a scintillation vial. In all cases, the maximum signal is achieved when the focal length is extended just beyond the glass interface. Figure 42 illustrates glass interference while measuring an acetone solvent in a round bottom flask. As the distance from the flask is increased, glass interference is pronounced and eventually overwhelms the spectrum.
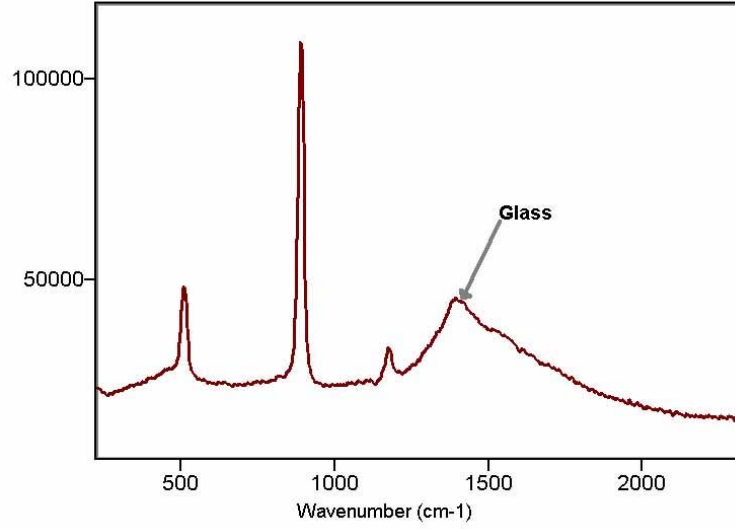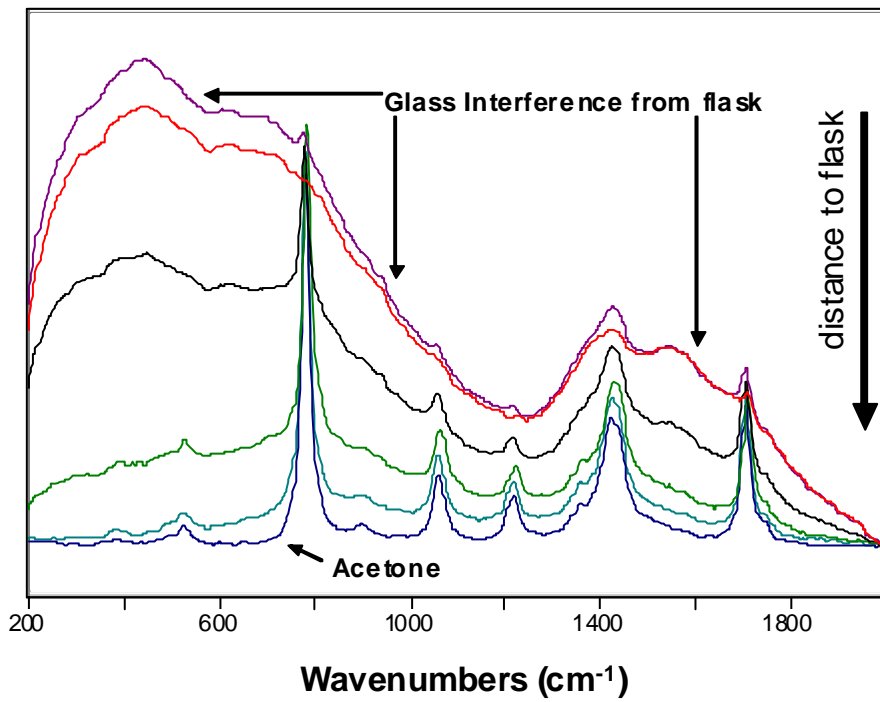
Fig. 41 (Glass in Spectra Example 785nm)

Fig. 42 (Round Bottom Flask Example 785nm)

10.0 Library Matching and Library Development

The NuSpec software comes with a basic library which is used to identify a wide variety of unknown samples. You can ask your sales representative for a recent Excel list of the library samples, or view them (as .spc or .dnu files) under the library directories.

The library builder software is used to create libraries for the spectrometer. All library entries are saved in a folder that you designate on your PC.

1) Open library builder software (**Fig 43**).
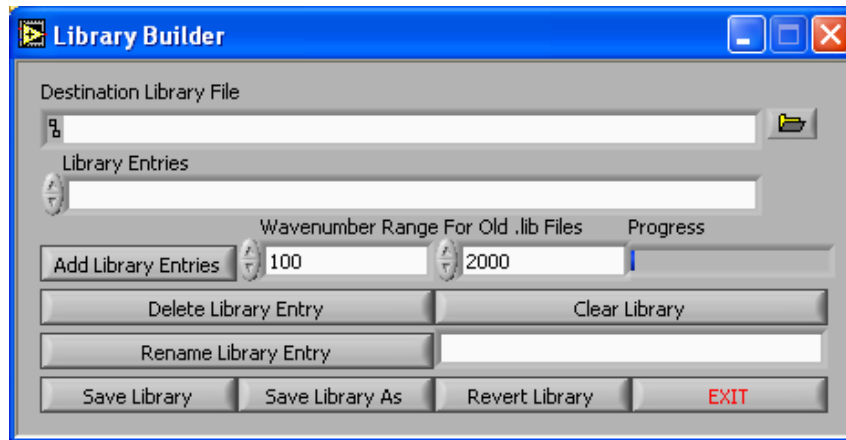2) Select the folder next to the top box to add the files that you wish to include in the new library.

Fig. 43 (Library builder software)

3) Once you have added your files you can select the file name to save your new library under using the folder next to the bottom box on the display window.
4) Click "Start".
5) The progress bar indicates the library development status.

To load up an existing library select the library under "destination library file", this loads that file and displays all the entries in the dropdown listbox selector. To delete, select the entry you want to delete in the dropdown listbox and click delete entry.

Note: When you load up an old format library (.lib file) you need to tell it the wavenumber range of the spectrometer that took those spectra in order for it to interpret the data correctly.

11.0 Sampling Attachments

The standard attachments for the Advantage are the Vial Sample Holder, Melting Point Tube Adapter, and NMR Tube Adapter. There are also several optional attachments for the Advantage series. The XYZ Stage, Extension Tube, Right Angle Input Optics, and the NuScope microscope.

11.1 Vial Sample Holder

The Vial Sample Holder comes standard with the instrument. It is mounted on the exit port with Allen screws and it is used for sampling liquids, emulsions and powders through 8mm vials. Inserts are provided for the analysis of liquids and crystals through NMR tubes and MP tubes. The focusing knob is used to optimize the Raman signal. The most intense Raman signal is obtained as the focal length is optimized (16.45 mm from the objective) just beyond the glass interface.
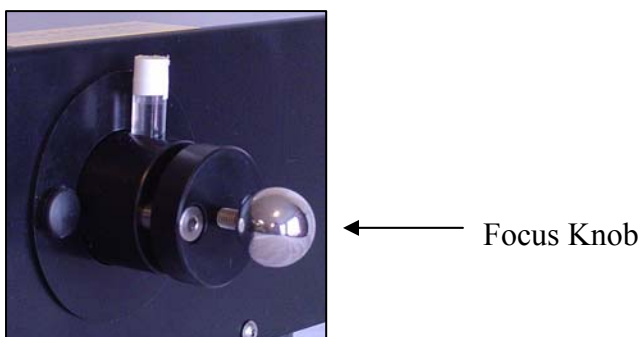

Focus Knob

Fig. 44 (Vial Sample Holder)

11.2 XYZ Stage

The XYZ Stage is a manual positioning stage mounted directly on the instrument near the face of the exit/collection port, which minimizes disturbances and vibrations that may occur with externally mounted stages. It is used to optimize and precisely position the beam on contaminants in thin films/coatings and semiconductors; inclusions in geological materials, and on biomedical substrates. The most popular method of securing the sample to the stage is two sided tape. The XYZ stage allows the user to position, traverse and adjust the focal position in micron steps.

Fig. 45 (XYZ Stage)

Steps To Attach XYZ Stage To Advantage System

1. MAKE SURE SYSTEM IS OFF!

3. Attach Magnetic Interlock Override With Magnets Facing Towards Advantage System

2. Remove Sample Holder Using 1/8" Allen Wrench

Magnetic Interlock Override

Sample Holder

4. Attach XYZ Stage To Bottom Of System Using 9/64" Allen Wrench

WITH MAGNETIC INTERLOCK OVERRIDE INSTALLED INSTRUMENT MAY EMIT CLASS 3A VISIBLE LIGHT
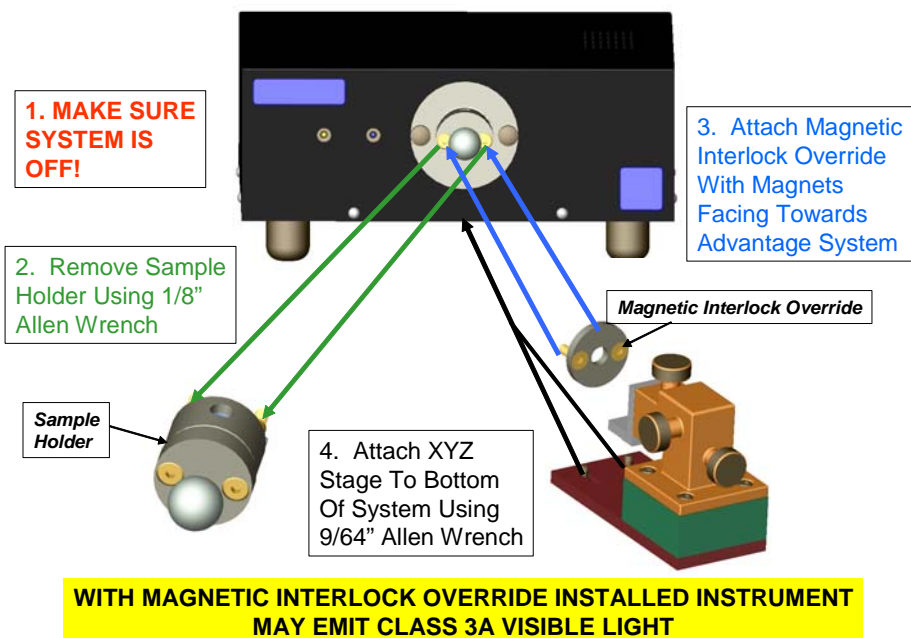
Fig. 46 (Attachment of XYZ Stage)

11.3 Right Angle Input Optics

The Right Angle Input Optics allows for horizontal sampling. This positions the exit/collection beam of the spectrometer at a 90 degree angle toward the sample. The focal length of the Right Angle Input Optics is approximately 16.45 mm.
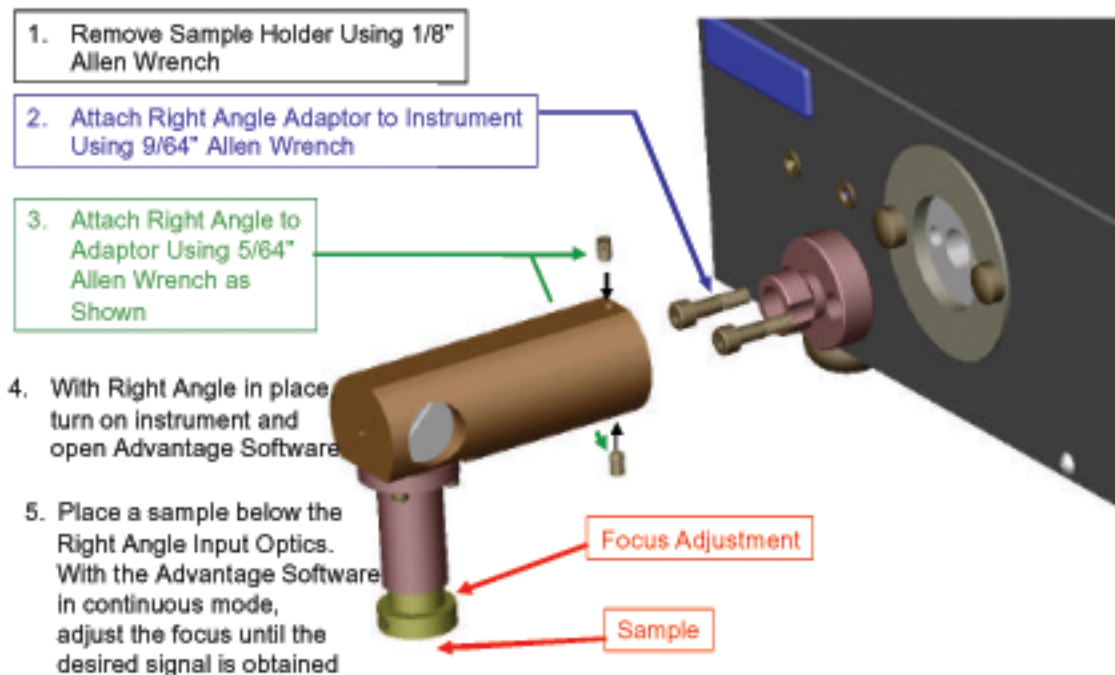
Fig. 47 (Right Angle Input Optics)

Fig. 48 (Attachment of Right Angle Input Optics)

11.4 Input Optics Extension

The Input Optics Extension incorporates objectives that extend the exit beam and collection signal 5 inches beyond the instrument. This allows the instrument to access a sample in congested locations. The focal point is approximately 16.45 mm beyond the Input Optics Extension.

Fig. 49 (Input Optics Extension)

11.5 NuScope

The Nuscope™ is a USB microscope attachment for imaging thin films, microelectronic circuits, mineral inclusions, and pigments in art work, identification of narcotics and plastic explosives, and biological tissues. The NuScope™ is attached with 7/64 Allen wrench. Illumination of the sample surface is obtained with external white light sources or ambient light. The surface image is viewed in real-time with a color video camera using USB Shot software, which allows movie and screen capture. Coarse focus is achieved on the specimen with manual adjustment, and fine focusing is obtained by a focus adjust knob to render a sharp image. Third party rack and pinion microscope stages are used to assist in focusing and transversing the sample across the field of view (FOV). The field of view is approximately 1000μm at 50x focal power.
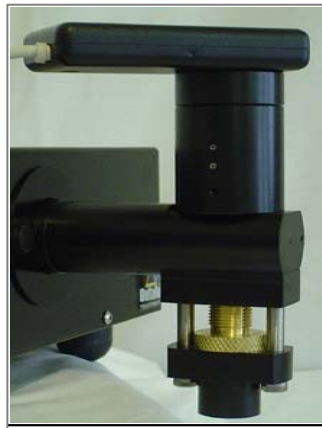

Fig. 50 (NuScope Attachment)

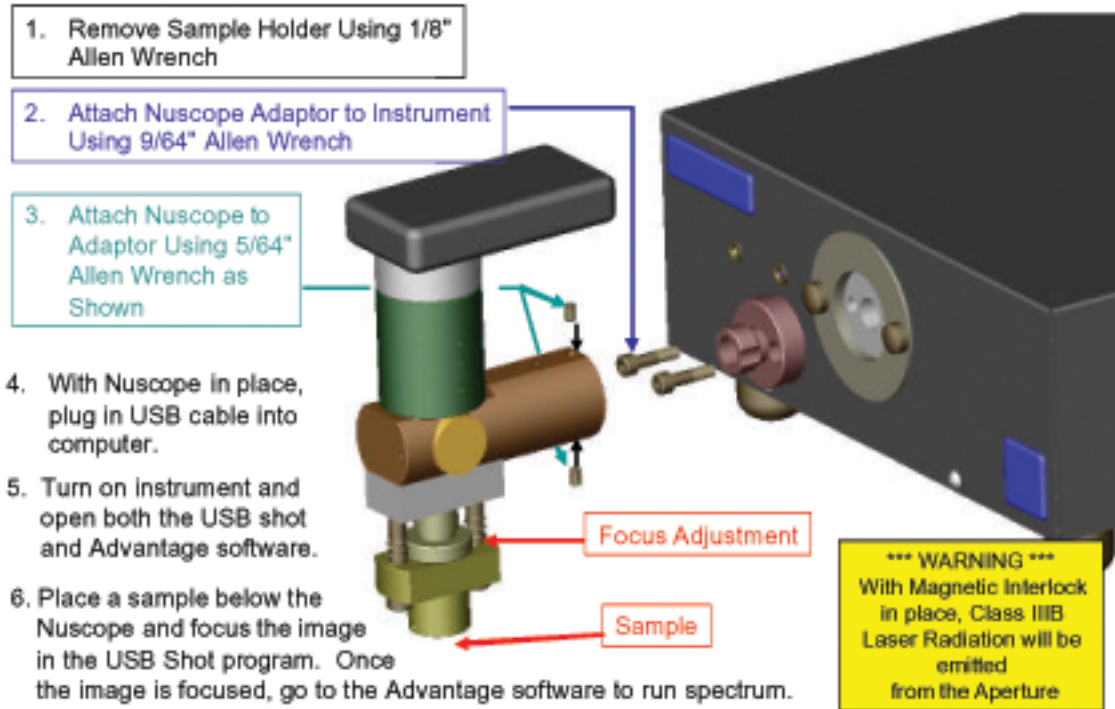*11.5.1 Installation of NuScope*

Fig. 51 (Installation of NuScope)

12.0 Loading the USB Driver

If your computer was not purchased with the spectrometer system the USB Drivers will need to be installed. To ensure that the USB Driver is properly installed please follow the steps below:
1) Connect the USB cable to the PC and to the spectrometer.
2) When prompted for on the *New Device Recognized* screen please browse for the file using automatic detection. The system will automatically search for the file **CDM 2.04.06 WHQL Certified** in the spectrometer software folder. If your PC is not able to detect the **CDM 2.04.06 WHQL Certified** folder automatically, you can select this file manually.

The first screen to appear is the *Welcome* screen (**Fig. 52**). **Select** "Yes, this time only" and click *Next*.
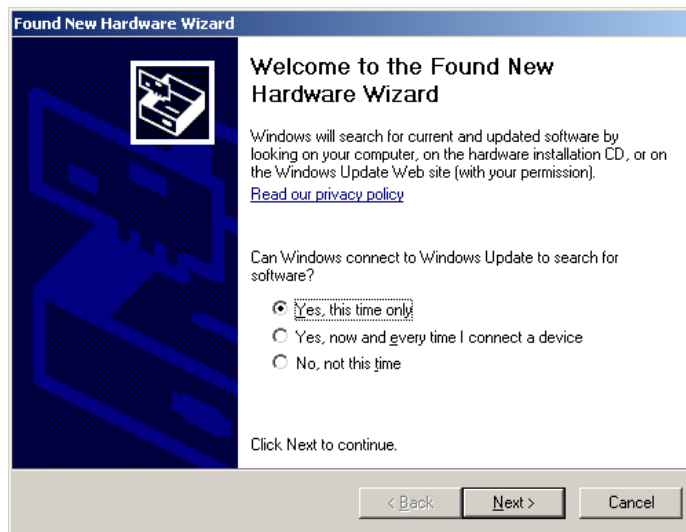

Fig. 52 (Welcome screen)

The next screen is the *USB High Speed Serial Converter* screen (**Fig. 53**). Select "Install from a list or specified location (Advanced)" and click *Next*.
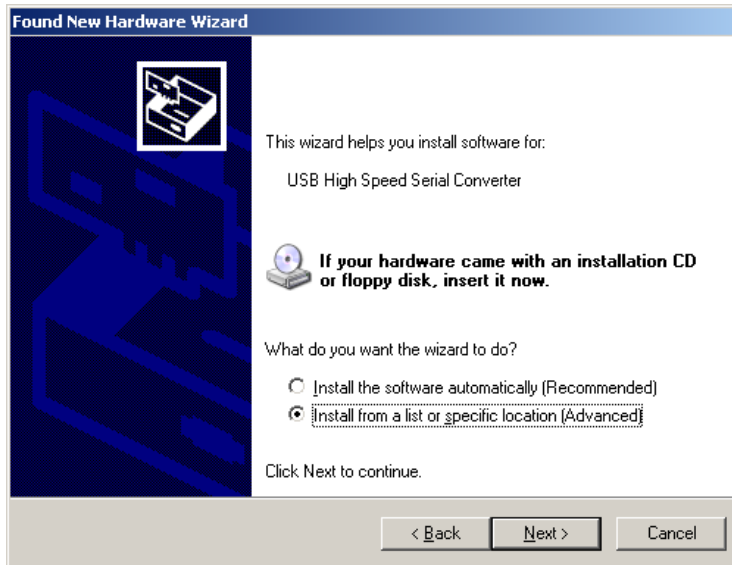
Fig. 53 (USB high speed serial converter screen)

The search and installation options menu will now appear.
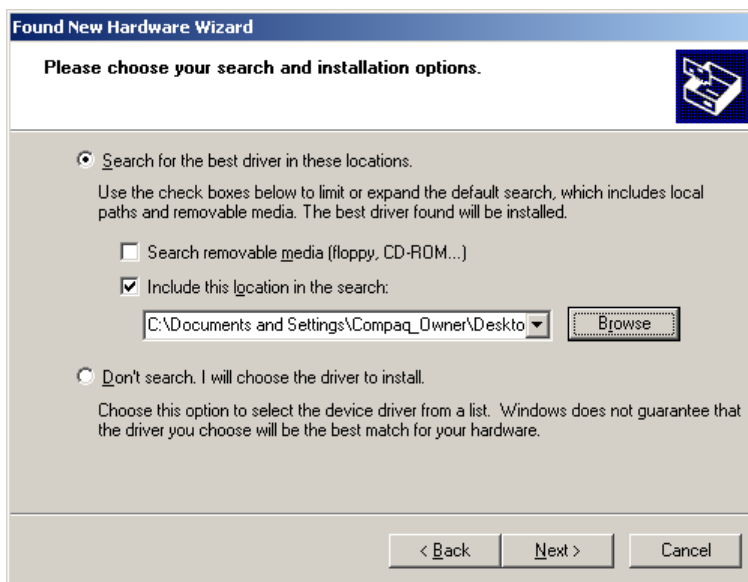
Fig. 54 (Search and installation options)

Select *Browse* and find the location of the USB Driver files. Click *Next* after you have selected the appropriate file location from the *Browse For Folder* window (**Fig. 55**).

Fig. 55 (Browse for folder window)

The software will now search for the driver files and install them. When it is finished the *USB Serial Converter* will be installed. Click **Finish**.

Fig. 56 (USB serial converter screen)

The second portion of the driver package will now be installed. Follow the same steps as above for the *USB Serial Converter*. This time you do NOT need to browse for the USB Driver file (it is now stored in the installer's memory). Upon completion you will see the *USB Serial Port* screen appear (**Fig. 57**). Click **Finish** and your spectrometer's USB link will be installed. You should only have to perform this installation once. However, if your computer contains multiple USB ports, it may be necessary to repeat this operation for every USB port that you intend to use for the spectrometer.
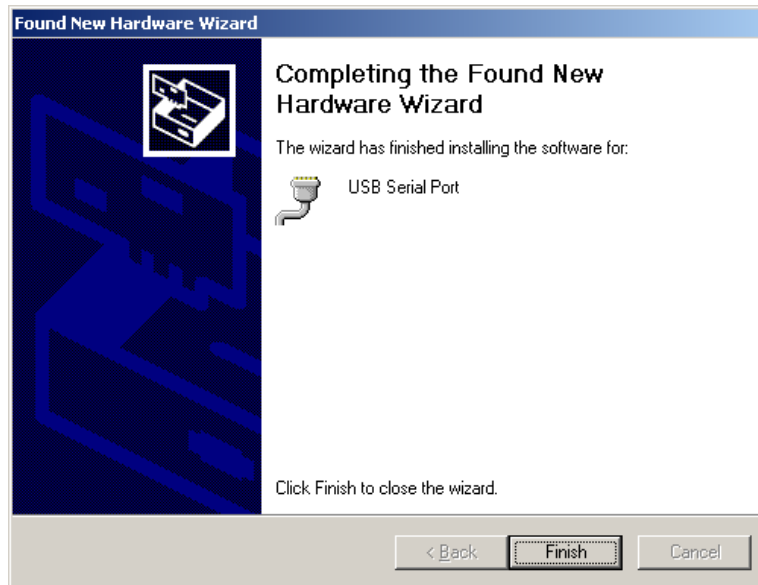

Fig. 57 (USB serial port screen)

13.0 Replacing the Desiccant

Extra desiccant containers come with your instrument. They will store dry in the containers provided. We recommend that the desiccant is changed every three months in humid areas, and every six months max in dryer areas. The desiccant will turn from a blue to pink when it has adsorbed a significant amount of moisture. If the desiccant is dark blue you do not have to change it.
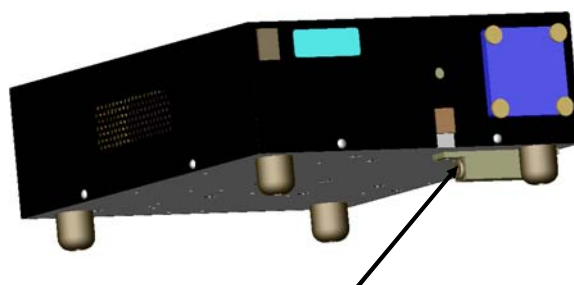
Figure 58 (Desiccant replacement instructions)

14.0 Raman Tutorial

Raman spectroscopy finds its origins in Planck and Einstein's formulation that light is not only wavelike in nature, but has the dual character of waves and particles. Once scientists began thinking about the concept of light as particles, the possibility of inelastic scattering of these particles became a method of proof of this new theory. In 1923 Compton showed this with inelastic scattering of x-rays from a graphite target. That same year Smekal theoretically predicted that photons should inelastically scatter from molecular transitions. Five years later, in 1928, C. V. Raman and K. S. Krishnan published an article in the journal Nature with experiments that proved Smekal's prediction of inelastic scattering of light. For his discovery, Raman was award the Nobel Prize and the inelastic scattering of visible light from molecular transitions has been named after him.

One of the characteristics of inelastic scattering is that the intensity of the scattering scales to the fourth power of the energy. This means that Compton's experiments with x-rays with a wavelength of 0.7 nm and the observation of Raman scattering

with visible light at 500 nm will differ by 11 orders of magnitude! Raman was able to observe the weak Raman effect by using the most intense light source available at the time, the sun. He focused a large telescope on the sun and placed a green filter in the intense beams of sunlight. When he used a yellow filter to observe this beam of green light passing through a solution of chloroform, he could see a weak yellow light. The origin of the yellow light was the Raman effect. A small amount of the green light from the sun had inelastically scattered from the chloroform molecules and shifted its energy so that the photons fell within the yellow part of the spectrum.

Today Raman spectroscopy has become much more sophisticated and much simpler to measure. Major technological advances have transformed Raman spectroscopy from telescopes and visual observation to a highly sensitive spectroscopic technique. The first technological advance was the laser. The sun is pretty bright, but it is sending its energy in all directions and over a very broad range of wavelengths. The Raman effect is observed as a shift in energy of a photon and the shift can be related to a vibrational state of the sample. To observe the shift one needs all of the photons to be a very narrow band of energies. Otherwise, you'll never be able to distinguish the shifted ones from the source photons. The laser produces quasi-monochromatic light that forms a very narrow band of frequencies. Lasers also produce this light in a small concentrated beam that is very intense.

A second technological advance that has revolutionized Raman spectroscopy is the Charge Coupled Device (CCD). The CCD is millions of times more sensitive than the eye and is able to record a complete Raman spectrum in less than a second. Let's look back at the history of Raman spectroscopy to understand the advantage that the CCD affords. Not long after Raman's first experiments scientists started using a photographic plate to record the Raman spectrum. This provided the first hardcopy of a Raman spectrum. The problem was that it didn't have a softcopy version that could be used for data analysis and spectral manipulation. Early Raman spectroscopy was also like shooting in the dark. The plates were exposed for hours to get a spectrum and you didn't know if the optics were properly aligned until the plate was developed. The next big advance was the photomultiplier tube. This is a sensitive optical transducer that converts photons into electrons. They are sensitive enough to detect a single photon and a whole technology of "single photon counting" grew around them. Now a Raman spectroscopist could set his instrument to view energy where Raman scattering should be observed and with an electronic display optimize the optical alignment. When computers became commonly available it was possible to have them record this electronic signal and produce a software file that contained the spectrum. This software file could be manipulated to remove artifacts that otherwise can distort Raman spectra.

However, the photomultiplier's advantage came at a cost. It was no longer possible to acquire a whole spectrum during a single integration period. These instruments would take very small steps in energy and count the photons at each step. A three

thousand point spectrum with photons counted for one second took three thousand seconds. That's almost an hour!

The solution was to go back to the old concept of a photographic plate, but this time use a digital camera. The CCD does just that. These little integrated circuit chips are extremely sensitive to light and they contain thousands of little picture elements (called pixels) that take the whole spectrum at once in less than a second.

Modern Raman spectroscopy with the Advantage is fun and educational. The Advantage system uses a small laser to replace Raman's telescope and colored filters, and a CCD detector to replace out-dated photomultiplier tubes. The result? A spectrum in seconds.

Intensity / Wavenumber (cm-1)

Polystyrene 785nm

Intensity / Wavenumber (cm-1)

Polystyrene 633nm

Intensity / Wavenumber (cm-1)

Polystyrene 532nm