RAMAN QUICK START GUIDE

IMSERC, Department of Chemistry, Northwestern University

Last Updated: 5/27/2014 by Saman Shafaie and Daniele Vinci

1. Starting up with FOM

Please log into the IMSERC FOM system. There is a terminal conveniently located in the ICP room nearby. Logging in will supply power to the instrument; the monitor is always on. If the computer on the Raman NIR has rebooted due to a power failure, log into the Fred4 account with the password "youshouldknow".

2. There are three types of sample holders: small glass vials, NMR tubes, and melting point tubes. ***Do not scan the instrument with an empty sample holder!***

3. Click on the NuSpec2 icon which is on the bottom-left corner of the desktop screen. This will start the software. The program will now ask which COM port to use. Choose COM 6. When the program starts, the yellow laser light on the front of the instrument will turn on. Click on the "Continuous" mode button and let the instrument warm up for a few minutes.

4. Abort the continuous scanning. At the top left, choose expert mode. Set the integration time to 1 second and the resolution to high. The laser power can be set to high. Set number of scans between references to 0.

6. Now place your sample. Use the appropriate insert in the sample holder if your sample is in an NMR or melting point tube.

7. Set the power, integration time, number of scans, and resolution for your sample. Low resolution usually gives the best results. Keep in mind that the laser beam is very intense and your sample could get hot and degrade during the scan time. Collecting 16 scans is usually sufficient.

8. You can print a simple report to the printer in the ICP lab. Wavelengths can be viewed by moving the cursor on the display.

9. You can save your results in several modes. The .dnu files can be loaded back up in the NuSpec2 software to view again. The .prn files are ASCII files that can be imported into Excel and then graphed using the Excel charting functions. For more advanced spectral manipulation, the list of wavelengths and intensities in the .prn file can be saved as a text file and then uploaded and manipulated with the OPUS software on the FTIR.

RAMAN in Depth GUIDE

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Spectra of liquids or solids in standard sample vials are obtained by placing the vial in the sample holder.

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.

←— Focus Knob
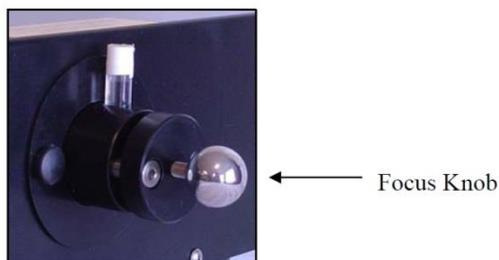
For liquids the focus is not critical, and there is a large range over which the adjustment makes very little change in the spectrum.

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.
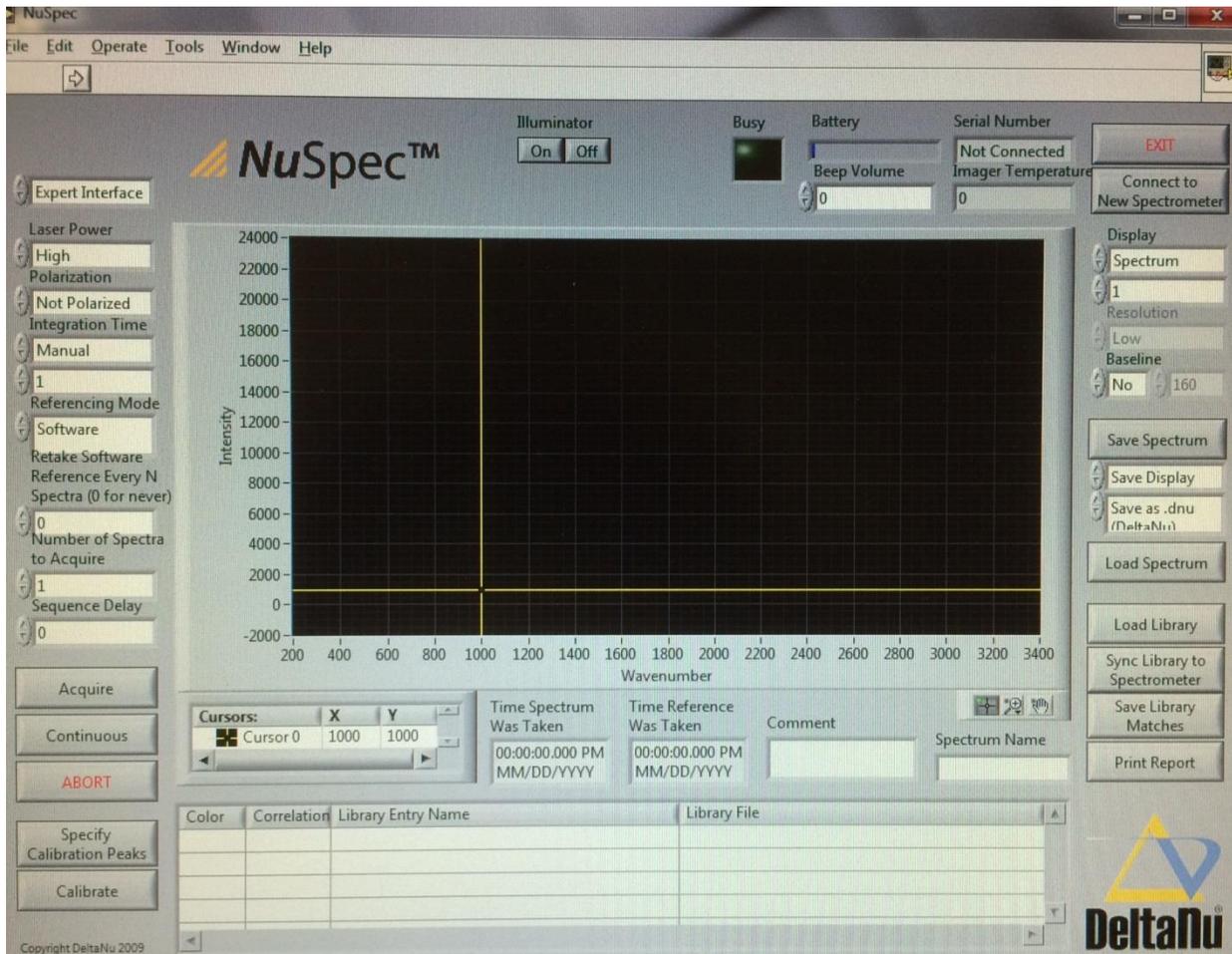
Laser Power

The *Laser Power* bar selects the power of the laser from five settings ranging from low to high. 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.

Polarization

This feature automatically subtracts molecule polarizations from the spectrum in order to provide information about the molecular symmetry. 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.

Integration time

The *Integration Time* feature has two modes: 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. 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.

Referencing Mode

"Software" referencing mode requires the manual *Integration Time* setting. 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.

Number of Spectra to Acquire

This feature is used to acquire a specified number of spectrum consecutively that can then be "averaged" together. 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.

Display

The *Display* feature allows the user to switch between showing the individual spectrum acquired or the "averaged" spectrum.

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.)

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.

Busy

The *Busy* light in the upper part 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.

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.

Abort

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

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.

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.

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. 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.

Load Spectrum

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

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 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.

The magnifying glass icon 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.
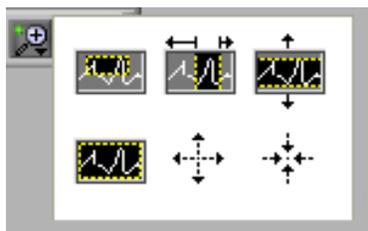
The cross icon allows the cursor to be moved to a specific location on the spectrum screen by clicking and dragging the cursor axis lines.
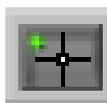
Print report

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.