Dimension Raman System

User Manual

DRS-RS1.4-UM-2010-10-26

Contents

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1 Introduction

This document describes the operation of the Dimension Raman systems, and the functionalities of its operating software RamanSoft.

Overview of Features

The Dimension Raman Systems are high performance Raman spectrometer systems achieved through the integration of proprietary optics and unique software solutions. The high sensitivity of its proprietary fiber bundle probe system and the high light throughput spectrometer, combined with unsurpassed performance provided by Princeton Instrument TE-cooled CCD, yield the sensitivity and resolution needed to confidently use Raman to answer critical QA/QC and analytical questions.

A Dimension-P Raman System comes with the LSI Vector Raman Probe, an internal sample cell (for Dimension-P1 Raman System), and an External Sampling Module. It is currently operated at 785 nm laser line. The system is available as a standard resolution (SR) (wavenumber coverage: 150 - 3300 cm^{-1}) or high resolution (HR) (150 - 2100 cm^{-1}; nominal resolution: 3 cm^{-1}) model.

A Dimension-M Raman Microscope System consists of a Dimension-P Raman System and the Raman Microscope Adaptor, which permits the user to convert a Nikon Model L-150 into a Raman microscope. The Nikon L-150 is an epi-illuminated reflectance microscope with infinity corrected optics allowing for analysis of surfaces as well as subsurface regions in translucent material. With a Dimension-M Raman Microscope system, the user can save both microscope images and Raman spectra of discrete sample regions and in the mean time make full use of the functionalities of the corresponding Dimension-P Raman system.

A Dimension Raman System is operated through RamanSoft, LSI's proprietary software for data acquisition, data processing, and data analysis for its Dimension Raman systems. This software functions as an instrument control center permitting user to set CCD temperature and integration time, control the laser shutter and laser power. In addition, RamanSoft has user-friendly interfaces for system calibration and system testing.

The program provides a comprehensive suite of data handling features designed to expedite data processing and analysis. These include several spectral smoothing algorithms for noise reduction, the ability to normalize spectra to different spectral features and/or to laser power, and LSI's proprietary algorithm for background removal to enable the user to obtain consistent spectra across different background conditions. These features along with quantitative procedures for peak intensity and peak area provide a full range of processing and analysis features.

RamanSoft is integrated with Thermo Electron Corporation's Spectral ID® for seamlessly performing spectrum acquisition and spectral library searching to identify unknown samples. The integrated system allows for setting experimental parameters, acquiring spectra to build a new library or add a spectrum to an existing library, and performing spectral identification of unknown samples.

RamanSoft is also integrated with Thermo Electron Corporation's IQ Predict™ for seamlessly performing spectrum acquisition and spectral analysis using both qualitative methods such as Principal Component Analysis and Discriminant Analysis, and quantitative methods like Principal Component Regression and Partial Least Squares analysis.

The Real Time Monitoring module is designed to meet the need to monitor processes in real time. The program tracks up to five peaks either by peak intensity or peak area, and can monitor processes as fast as 25 msec.

How This Document Is Organized

Manual Organization

This manual provides the user with all the information needed to set up and operate a Dimension Raman System.

- Chapter 1, “Introduction”, provides an overview of the Dimension Raman System.
- Chapter 2, “System Setup”, provides an overview of the hardware components, the instructions for setting up a Dimension Raman System, and the procedures for starting up and shutting down the instrument.
- Chapter 3, “Installing RamanSoft”, gives a detailed description on how to install the software on your computer.
- Chapter 4, “A Quick Tour”, gives a quick tour of the operation and functionality of the Dimension Raman System.
- Chapter 5, “RamanSoft Basic Functionalities”, details the basic functionalities of RamanSoft, which includes data acquisition, data processing, and data analysis.
- Chapter 6, “The SpectrumSearch Module”, details the RamanSoft SpectrumSearch functionality in creating a spectral library, performing online one-step spectrum acquisition and library searching or off-line spectral library searching of an existing spectrum through the integrated SpectrumSearch interface for Thermo Electron Corporation’s Spectral ID®.
- Chapter 7, “The SpectrumPredict Module”, details the RamanSoft SpectrumPredict functionality in performing one-step spectrum acquisition and qualitative and/or quantitative spectrum prediction through the integrated SpectrumPredict interface for Thermo Electron Corporation’s IQ Predict™.
- Chapter 8, “The Real Time Monitoring Module”, details the RamanSoft functionality in monitoring a process in real time.
- Chapter 9, “Virtual RamanSoft”, describes the operation of RamanSoft independent of the operation of a Dimension Raman System.
- Appendix I, “System Specifications’.
- Appendix II, “Mercury Lamp Peaks for Wavelength Calibration”.
- Appendix III, “The Maximum Peak of Cyclohexane for Laser Wavelength Calibration”.
- Warranty & Service contains the warranty and customer support contact information.

Safety Related Symbols Used in this Manual

Caution! The use of this symbol on equipment indicates that one or more nearby items should not be operated without first consulting the manual. The same symbol appears in the manual adjacent to the text that discusses the hardware item(s) in question.

Warning! Risk of electric shock! The use of this symbol on equipment indicates that one or more nearby items pose an electric shock hazard and should be regarded as potentially dangerous. This same symbol appears in the manual adjacent to the text that discusses the hardware item(s) in question.

Caution! The use of this symbol on equipment indicates that the laser should not be operated without first consulting the manual. The same symbol appears in the manual adjacent to the text that discusses the hardware item(s) in question.

Optional Documents

GRAMS/AI™ User's Guide, PLSplus IQ™ User's Guide, and Spectral ID® User's Guide from Thermo Electron Corporation.

System Safety

Grounding and Safety

Before plugging in or turning on power supply, the ground prong of the power cord plug must be properly connected to the ground connector of the wall outlet. The wall outlet must have a third prong, or must be properly connected to an adapter that complies with these safety requirements.

WARNING! If the equipment is damaged, the protective grounding could be disconnected. Do ***not*** use damaged equipment until its safety has been verified by authorized personnel. Disconnecting the protective earth terminal, inside or outside the apparatus, or any tampering with its operation is also prohibited.

Inspect the supplied power cord. If it is not compatible with the power socket, replace the cord with one that has suitable connectors on both ends.

WARNING! Replacement power cord or power plugs must have the same polarity as that of the original ones to avoid hazard due to electrical shock.

Precautions

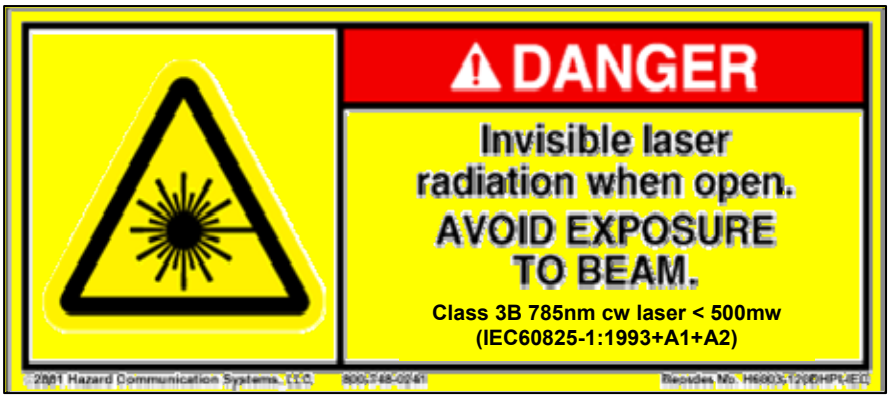
To prevent permanently damaging the system, please observe the following precautions:

- Do not remove the instrument cover without turning the system power off and disconnecting the power cord and USB cable. In general, disconnecting cables inside the instrument is not recommended.
- If you are using high-voltage equipment (such as an arc lamp) with the Dimension Raman system, be sure to turn the system power ON LAST and turn the power OFF FIRST.
- Use caution when triggering high current switching devices (such as an arc lamp) near your system. The CCD can be damaged by transient voltage spikes. If electrically noisy devices are used nearby, an isolated, conditioned power line or dedicated isolation transformer is highly recommended.
- The Laser Fiber and Signal Fiber cables should be placed to allow them to follow their natural curvatures. Bending or pressing the Laser cables on sharp edges or angles could have them permanently damaged.
- Do not place liquids or solvents on top of the Dimension-P Raman unit.
- Do not store unit at temperatures above 50°C.
- Do not operate unit for more than one hour continuously at ambient temperatures above 40°C.
- Never prevent the free flow of air through the equipment by blocking the air vents and fan vent of the Dimension-P Raman unit.
- Allow ambient room around the Dimension-P Raman unit and place the unit in such a way that it is easy to unplug the power cord from the back of the Dimension-P Raman unit to remove power to the unit in case of a fire hazard.

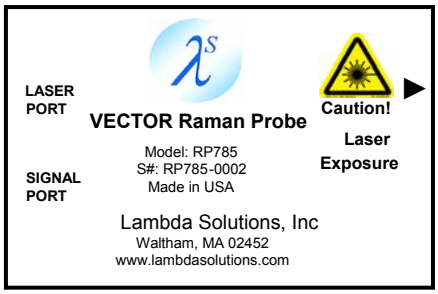
Laser Safety

The system employs a Class IIIB 785 nm (see labels below) diode laser (please refer to system specifications in Appendix I for laser-related parameters) for excitation. This wavelength is not as sensitive to human eyes as visible light. Therefore, the laser beam is more powerful than you see! Direct eye contact or prolonged exposure to the skin may cause injury and therefore should be avoided.

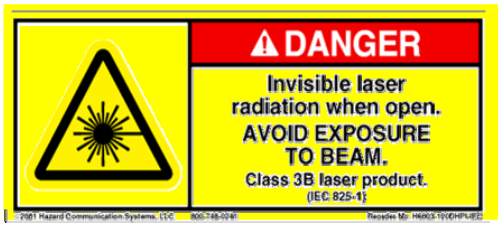
Laser safety label:



Raman probe label:



Access panel label:



Before the laser power key switch on the Dimension-P Raman unit is turned on, one end of the laser fiber should be firmly attached to the laser aperture (i.e., Laser Out port) on the front panel of the Dimension-P Raman unit, and the other end should be firmly connected to the Laser Port of the Vector Raman Probe (or of the Raman Microscope Adaptor). Place the Vector Raman Probe in the internal sample cell or the External Sampling Module and make sure to close the lid.

Before operation, make sure that the laser emission aperture is not pointing towards anyone's eye, or onto any surface that may cause reflection into anyone's eye. During operation, avoid direct viewing of the laser beam or any specular reflection of the laser beam. To lower the risk of injury to the eye, make sure everyone in the room is wearing laser safety goggles while the laser is operating.

Turn the Laser Key to OFF position on Dimension-P Raman unit if the laser fiber needs be detached or reconnected. Detach or reconnect the fiber while the laser is on could injure people.

Do not remove internal laser housing compartment.

Caution! Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Interlock:

The system is also equipped with an interlock in the back of the instrument panel (see figure below for P1, its exact position on the back panel for P2 is slightly different from the figure). The user is recommended to connect the interlock to his/her lab laser safety warning system according to his/her lab safety requirements.

The interlock is set as follows. When the pin 1 and pin 6 of the 6-pin phone plug is connected (shorted), the laser power is allowed to be turned on/off by the laser power key switch. If the key is turned on, the green LED in the front panel is on. When it is not connected (open) the laser power cannot be turned even if the key switch is turned on.

System Maintenance

Cleaning of the Optical Surfaces

WARNING! Turn off all power to the equipment and secure all covers before cleaning the units. Otherwise, damage to the equipment or injury to you could occur.

Optical surfaces may need to be cleaned due to the accumulation of atmospheric dust. We advise that the *drag-wipe* technique be used. This involves dripping a clean cellulose lens tissue into clean anhydrous methanol, and then dragging the dampened tissue over the optical surface to be cleaned. Do not allow any other material to touch the optical surface.

2 System Setup

To minimize risk to users or to system components, turn the Dimension Raman unit OFF before any cables are connected or disconnected.

System Components

Upon unpacking, check that you have all the components to set up a Dimension Raman system. A complete system consists of:

- Dimension-P1 Raman or Dimension-P2 Raman
- Vector Raman Probe
- External Sampling Module
- Laser Fiber (thin black cable)
- Signal Fiber (thick black cable)
- Power cord for Dimension-P Raman
- Laser Power Key
- Sealed cyclohexane standard sample
- USB 2.0 cable
- RamanSoft CD-ROM
- Dimension Raman System User Manual
- Internal Sample Cell Handle (for switching sample compartments of the internal sample cell of Dimension-P1 Raman)
- (Optional) Microscope, Raman Microscope Adaptor, Calibration Kit
- (Optional) Additional Software - GRAMS/AI™ 7.0 with PLSplus/IQ add-on (or IQ Predict™), Spectral ID®, and their corresponding user manuals.

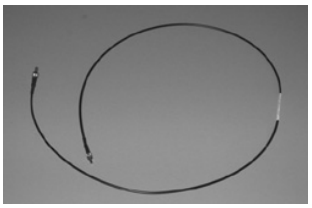
Standard Components

Dimension-P1 Raman

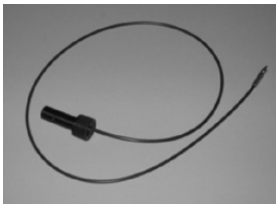
OR

Dimension-P2 Raman

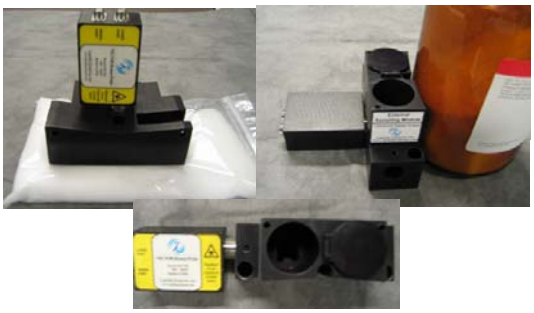
Vector Raman Probe



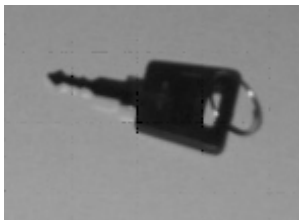
Laser Fiber



Signal Fiber

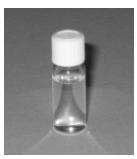


External Sampling Module



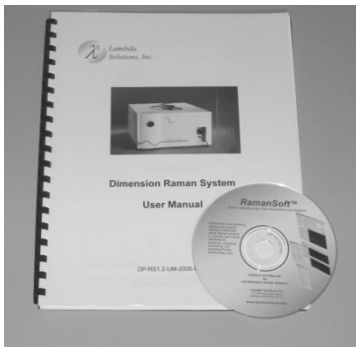
Internal Sample Cell Handle
(for Dimension-P1)
and Laser Power Key

Dimension Raman-P
Power Cord



Sealed Cyclohexane
Standard Sample

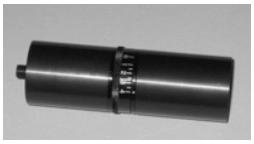
USB 2.0 Cable



Dimension Raman System

User Manual and RamanSoft CD-ROM

Optional Components



Calibration Kit

Raman Microscope Adaptor
(microscope not shown)

Thermo GRAMS software
& manuals

System Requirements

Environmental Requirements

Storage temperature: ≤ 55°C

Operating environment temperature: 5°C to +30°C; the environment temperature range over which system specifications can be guaranteed is +18°C to 23°C.

Relative humidity ≤ 50%; non-condensing

Note: For TE-cooled cameras, the cooling temperature may degrade if the room temperature is above +23°C.

Ventilation

Allow at least one inch clearance for the side air vents and at least three inches clearance for the back fan vents of the Dimension Raman spectrometer.

Power

Power Input: AC 100 – 250 V, 2 – 4 Amps, 50/60 Hz
Fuse: 250 V, 2 A, 5 x 20 mm
Max Power Consumption:
Dimension-P1 Raman: 95 W
Dimension-P2 Raman: 75 W

Computer and USB 2.0 Protocol

- Computer with Pentium 3 processor, or better, at 1 GHZ or above.
- Windows® XP (with Service Pack 1), Windows® 2000 (with Service Pack 4) or later operating systems.
- Native USB 2.0 support on the mother board or USB Interface Card (orange Micro 70USB90011 USB2.0 PCI is recommended for desktop; SIIG, Inc. USB 2.0 PC card, Model US2246 for laptop).
- Minimum of 256 MB of RAM.
- CD-ROM drive.
- Hard disk with a minimum of 10 GB available. A complete installation of the program files takes about 20 MB.
- Supper VGA monitor and graphics card supporting at least 256 colors with at least 1 MB of memory. Memory requirement is dependent on desired display resolution.
- Mouse or other pointing device.

Setting up a USB 2.0 Interface

Administrator privileges are required under Windows 2000 and Windows XP to install RamanSoft. The advantages of the USB2.0 interface are that it uses a much higher data transfer rate than many common serial data formats and it simplifies the connection to external devices. USB supports “Plug and Play”, i.e., the user does not need to be heavily involved in the setup process.

USB 2.0 Limitations

- Maximum cable length is 5 meters (16.4 feet)
- 1 MHz is currently the upper digitization rate limit for PIXIS™ camera. Large data sets and/or long acquisition times may be subject to data overrun because of host computer interrupts during data acquisition.

Note: If you are installing the USB 2.0 interface on a laptop, you need to perform all of the operations described in this section. In addition, if you are using the recommended USB Interface Card (USB 2.0 PC Card, Model US2246, SIIG, Inc.), you must replace the OrangeUSB USB 2.0 Host Controller driver installed for that card with the appropriate Microsoft driver.

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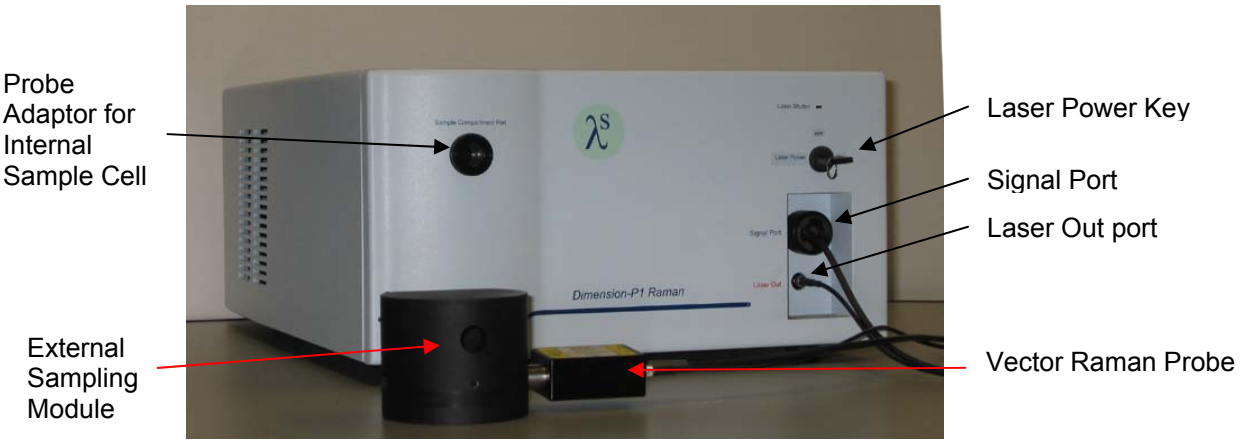
Updating the OrangeUSB USB 2.0 Driver

This procedure is highly recommended when a laptop computer will be used to communicate with the PIXIS™ camera. As stated before, we recommend the USB 2.0 PC Card, Model US2246 from SIIG, Inc. if USB 2.0 is not native to the laptop's motherboard.

1. Download and install Microsoft Service Pack 4 (for Windows 2000) or Service Pack 1 (for Windows XP) if the service pack has not been installed.
2. From Windows *Start* menu, select *Control Panel*.
3. Select *System* and then *System Properties*.
4. Select the *Hardware* tab and click on *Device Manager* button.
5. Expand *Universal Serial Bus Controllers*.
6. Right-mouse click on *OrangeUSB USB 2.0 Host Controller* and select *Properties*.
7. On the *Driver* tab, click on the *Update Driver*… button. You may have to wait a minute or so before you are allowed to click on the button.
8. When the *Update Device Driver Wizard* appears, click on *Next*. Select the *Search for a suitable driver…* radio button.
9. On the next screen select the *Specify a Location* checkbox.
10. Browse and select the location. Click on *OK*.
11. In the *Driver Files Search Results* window, check the *Install one of the other drivers* checkbox.
12. Select the *NEC PCI* to *USB Enhanced Host Controller B1* driver. Click on *Next* and the installation will take place. When the *Completing the Upgrade Device Driver Wizard* window appears, click on *Finish*. You will then be given the choice of restarting the computer now or later. The hardware associated with the driver will not work until you restart computer.

Setting up the Dimension-P1 Raman System

Unplug or switch off the power switch of Dimension-P1 Raman and the computer before connecting or disconnecting the USB 2.0 cable.



1. Connect the thin black Laser Fiber to the **Laser Out** port, and the Fiber Slit Assembly end of the thick black Signal Fiber to the **Signal Port** on the lower right front panel of Dimension-P1 Raman.

> **Note**: Make sure that the two set screw holes on the Fiber Slit Assembly face upwards and that the screw mechanisms for both the Laser Fiber and Signal Fiber are finger tight for proper focus.

2. Connect the other end of the Laser Fiber and Signal Fiber to the **Laser Port** and the **Signal Port** on the Vector Raman Probe, respectively.

> **Note**: Make sure that the screw mechanisms for both the Laser Fiber and Signal Fiber are finger tight for proper focus.

3. If using the internal sample cell, unscrew loosely the Probe Adaptor on the upper left corner of Dimension-P1 Raman. Insert the Vector Raman Probe into the **Sample Compartment Port** through the Probe Adaptor. Make sure that the Vector Raman Probe is inserted till the probe head touches the end of the Sample Compartment Port. Screw finger tight the Probe Adaptor.

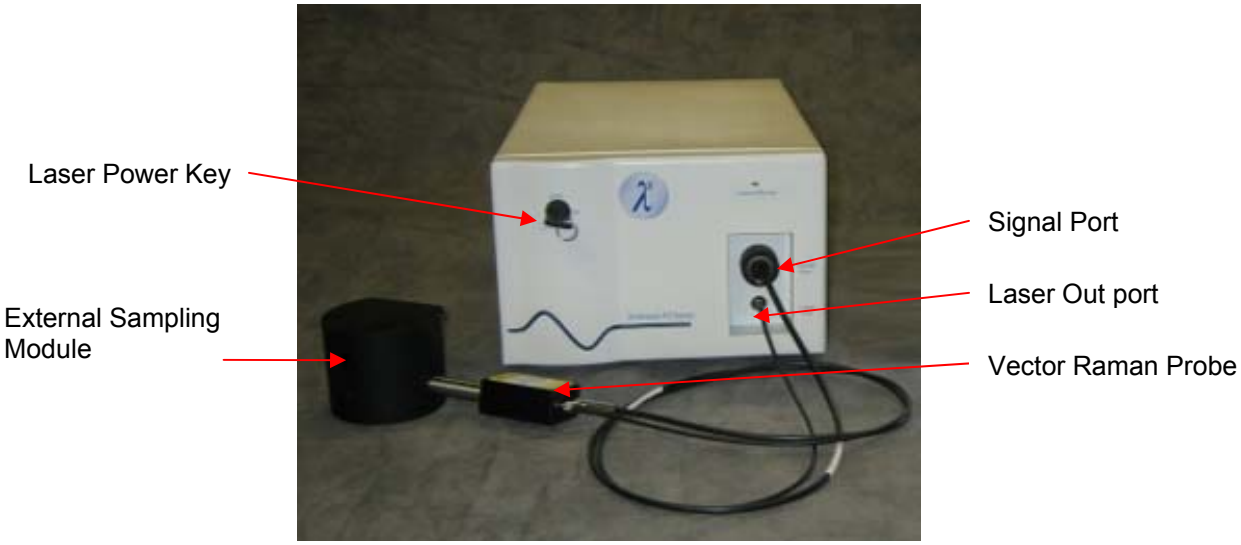
   If using the External Sampling Module, place the Vector Raman Probe into the desired probe hole on the External Sampling Module. Make sure to tighten the screw in the screw hole of the External Sampling Module so that the Vector Raman Probe is in leveled position (instead of being tilted due to the weight of the probe head).

4. Connect the Dimension-P1 Raman (USB 2.0 port at the back panel of the unit) to your computer with the USB 2.0 cable.

5. Plug in the power cord of the Dimension-P1 Raman and of the computer, respectively.
6. Insert the Laser Power Key into the Laser Power keyhole on Dimension-P1 Raman. Leave it in **OFF** position.

Setting up the Dimension-P2 Raman System

Unplug or switch off the power switch of Dimension-P2 Raman and the computer before connecting or disconnecting the USB 2.0 cable.



1. Connect the thin black Laser Fiber to the **Laser Out** port, and the Fiber Slit Assembly end of the thick black Signal Fiber to the **Signal Port** on the lower right front panel of Dimension-P2 Raman.

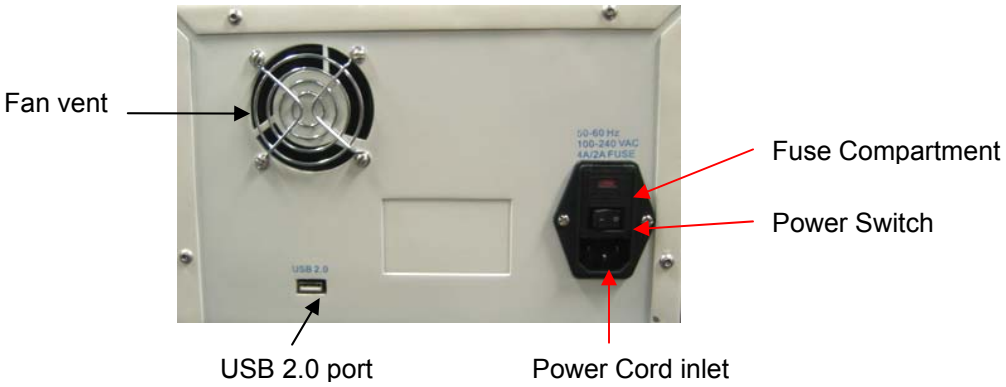
> **Note**: Make sure that the two set screw holes on the Fiber Slit Assembly face upwards and that the screw mechanisms for both the Laser Fiber and Signal Fiber are finger tight for proper focus.

2. Connect the other end of the Laser Fiber and Signal Fiber to the **Laser Port** and the **Signal Port** on the Vector Raman Probe, respectively.

> **Note**: Make sure that the screw mechanisms for both the Laser Fiber and Signal Fiber are finger tight for proper focus.

3. Place the Vector Raman Probe into the desired probe hole on the External Sampling Module. Make sure to tighten the screw in the screw hole of the External Sampling Module so that the Vector Raman Probe is in leveled position (instead of being tilted due to the weight of the probe head).

4. Connect the Dimension-P2 Raman (USB 2.0 port at the back panel of the unit) to your computer with the USB 2.0 cable.



5. Plug in the power cord of the Dimension-P2 Raman and of the computer, respectively.

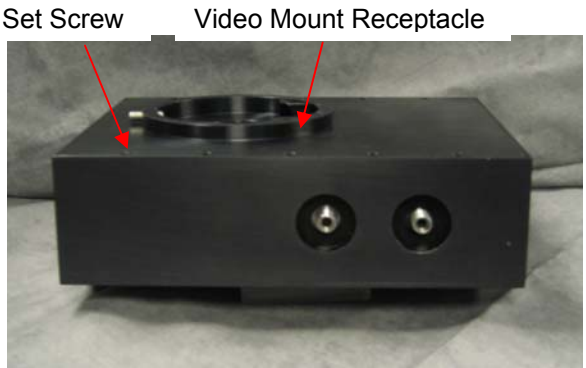
6. Insert the Laser Power Key into the Laser Power keyhole on Dimension-P2 Raman. Leave it in **OFF** position.

Setting up the Dimension-M Raman Microscope System

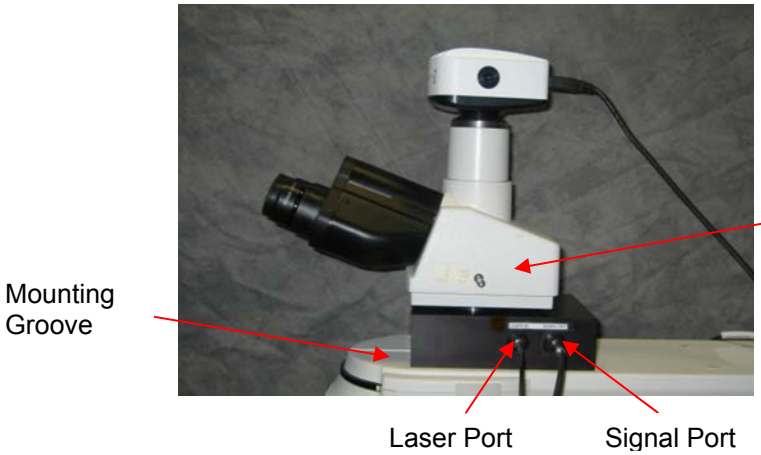
Unplug or switch off the power switch of Dimension-P Raman, the Nikon L-150 Epi Microscope, and the computer before connecting the USB 2.0 cable.

1. Mounting the Raman Microscope Adaptor

    a. Place the Raman Microscope Adaptor on the microscope. The Video Mount Receptacle of the adaptor should match the mounting receptacle on the microscope.



b. Tighten the adaptor in place with a 5/64 Allen-wrench or hex driver by inserting the wrench in the mounting groove.



2. Mounting the Infinity 1 CCD Camera (or camera of your choice)

   a. Place the Video Mount on the Video Mount Receptacle of the adaptor, tighten with the Set Screw.

   b. Install the C mount-lens which comes with the Infinity 1 CCD camera (or a camera of your choice).

3. Connect the thin black Laser Fiber to the **Laser Out** port, and the Fiber Slit Assembly end of the thick black Signal Fiber to the **Signal Port** on the lower right front panel of Dimension-P Raman.

   Note: Make sure that the two set screw holes on the Fiber Slit Assembly face upwards and that the screw mechanisms for both the Laser Fiber and Signal Fiber are finger tight for proper focus.

4. Connect the Laser Fiber to the **Laser Port**, and Signal Fiber to the **Signal Port**, respectively, of the Raman Microscope Adaptor.

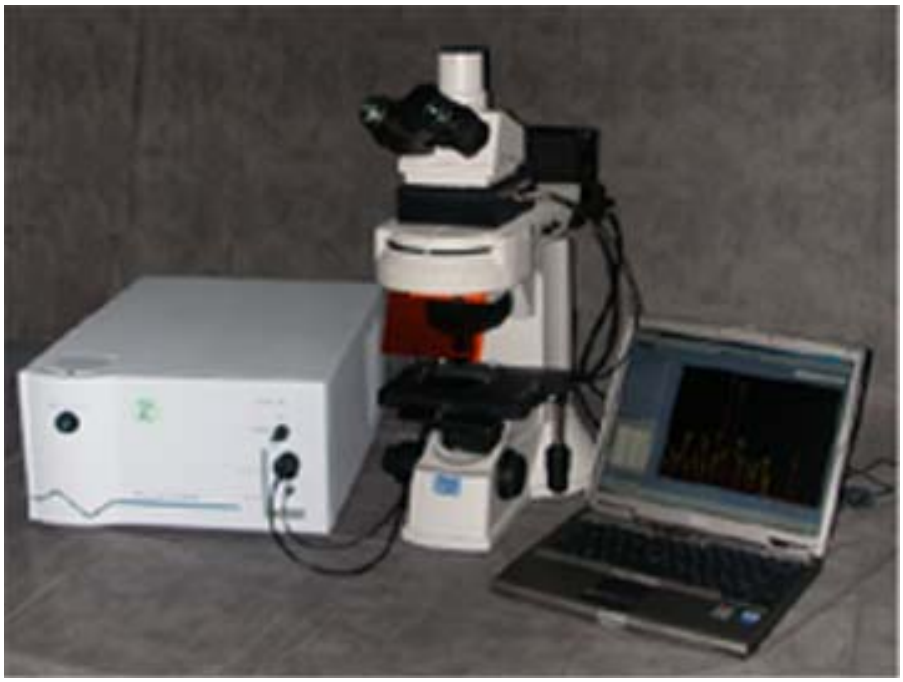
   Note: Make sure that the screw mechanisms for both the Laser Fiber and Signal Fiber are finger tight for proper focus.

5. Connect the Dimension-P Raman (USB 2.0 port at the back panel of the unit) to your computer with the USB 2.0 cable.

7. Plug in the power cord of the Dimension-P Raman, the Nikon L-150 Epi Microscope, and of the computer, respectively.

8. Insert the Laser Power Key into the Laser Power keyhole on Dimension-P Raman. Leave it in **OFF** position.

9. The following figure shows an assembled Dimension-M1 Raman Microscope system (Dimension-P1 Raman with Raman Microscope Adaptor and Nikon L-150 Epi Microscope. Note: the computer shown in the picture is not included in the system.)



System Start-up Procedure

- Turn on the power of the Dimension-P Raman (the power switch is on the back panel of the unit).
- Turn the Laser Power Key to **ON** position.
- Turn on the computer and launch RamanSoft. If RamanSoft is not installed on your computer yet, please do so by following the instructions in Chapter 3, “Installing RamanSoft”. Also, install optional software (such as Thermo Electron Corporation’s software).
- For a Dimension-M Raman Microscope system, turn on the microscope lamp. Also, install the software that comes with the Infinity 1 CCD camera (or a camera of your choice).

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Using the Dimension-P Raman System

- Place a sample, in vial or cuvette, in the appropriate sample compartment in the internal sample cell or the External Sampling Module. Make sure to close the lid of the internal sample cell or the External Sampling Module. If a surface or object is to be analyzed, place the External Sampling Module on top of the sample and insert the Vector Raman Probe downwards into the large round hole till the probe head touches the stopper of the External Sampling Module.

- Make sure the Laser Power key is at the **ON** position.
- Set data acquisition parameters to acquire spectrum (Acquire or Continuous Acquire, accessed through RamanSoft main menu *Acquisition->Acquire* or *Acquisition->Continuous Acquire*, or by clicking on icon  or  , respectively, from the RamanSoft Acquisition Toolbar) and access RamanSoft function modules as described in Chapter 4, “A Quick Tour”. For Detailed functionalities, see Chapters 5 -8.

Using the Dimension-M Raman Microscope System

- Focus the laser beam. To focus the beam, the user must visualize the beam with the Infinity 1 CCD Camera (or the camera of your choice).
    a. Place a sample on the microscope stage.
    b. Start RamanSoft and set the laser power to 30 mW (see Chapter 4, “A Quick Tour”). This low setting is required so as not to over-saturate the Infinity 1 CCD Camera and therefore be able to obtain a true assessment of optimal (i.e., smallest diameter) beam size.
    c. Start the Infinity 1 Live Video Capture software. Use default setting for frame size and resolution.
    d. Toggle back on to RamanSoft and start continuous acquisition (accessed through RamanSoft main menu *Acquisition->Continuous Acquire*, or by clicking on icon  from the RamanSoft Acquisition Toolbar. See Chapter 4, “A Quick Tour”).
    e. The beam can be seen on live video. Adjust the focus until the laser beam is the smallest.
    f. Turn off continuous acquisition by pressing the  button on the RamanSoft Acquisition Toolbar (See Chapter 4, “A Quick Tour”).
- Visualize the sample. Locate the region of interest.
    a. Turn on the microscope lamp and adjust the microscope stage until the field of interest is in the view.
    b. Turn the microscope lamp to the lowest setting that still allows visualization of the sample.
    c. Start continuous acquisition and make sure that the sample is centered under the beam.
    d. Turn off the microscope lamp and re-focus the laser beam if necessary.
    e. Turn off continuous acquisition.
- Acquire sample spectrum
    a. Increase the laser power and restart continuous acquisition.

b. Optimize other acquisition parameters (See Chapter 5, RamanSoft Basic functionalities) by taking and comparing spectra obtained at different acquisition parameter settings.

c. Acquire spectrum (accessed through RamanSoft main menu *Acquisition->Acquire* or by clicking on icon  from the RamanSoft Acquisition Toolbar) and access RamanSoft function modules as described in Chapter 4, "A Quick Tour". For Detailed functionalities, see Chapters 5 -8.

Switching between Macro-sampling and Micro-sampling

The difference between the Dimension-P Raman System and the Dimension-M Raman Microscope System lies in the different sampling method. For a Dimension-P Raman System, the sampling is performed using the External Sampling Module, and in the case of Dimension-P1, an additional internal sample cell is provided; the size of the laser beam is approximately 0.2 mm, and such sampling is termed as macro-sampling. For a Dimension-M Raman Microscope System, the sampling is performed using the microscope stage, where the microscope objective can focus the laser beam to 20 – 100 μm with current multimode laser fiber (when a single mode laser fiber is used, the laser spot will be focused smaller), and thus sampling can be performed on even smaller distinct sub-regions of the sample. Such sampling is termed micro-sampling.

The user is able to switch between macro-sampling and micro-sampling during a RamanSoft session. To do this, proceed with the following procedure:

1. Check whether there is a continuous acquisition currently active, if so, click on *Acquisition -> Stop Acquire* or press  to stop the continuous acquisition.

2. Turn the Laser Power Key on the Dimension-P Raman to **OFF** position.

3. Disconnect the Laser Fiber and the Signal Fiber from the **Laser Port** and the **Signal Port** of the Vector Raman Probe (or Raman Microscope Adaptor), respectively, and connect them to the corresponding port of the Raman Microscope Adaptor (or Vector Raman Probe), respectively.

   Note: Make sure that the screw mechanisms for both the Laser Fiber and Signal Fiber are finger tight for proper focus.

4. Turn the Laser Power Key on Dimension-P Raman to **ON** position.

5. Follow the procedure in "Using the Dimension-M Raman Microscope System" (or "Using the Dimension-P Raman System") to proceed with the switched sampling system.

System Shut-down Procedure

- Exit each of the RamanSoft function modules, if used, and then exit RamanSoft. Turn off the computer.

- Turn the Laser Power key to the **OFF** position.

- Turn off Dimension-P Raman (power switch to “0” position on the back panel).
- Turn off the microscope lamp if Dimension-M Raman Microscope is used.

Trouble Shooting

Do not open unit without taking safety precautions! (see Chapter 1, section “System Safety”).

How to Replace Fuse

If the Dimension-P Raman failed to turn on (as indicated by the absence of sound from the fan) after the power cord is plugged in and the power switch is at “1” position, the fuse(s) may need be replaced.

The AC power in-let module on the back panel of the system has a fuse compartment containing two fuses. To replace one or both fuses, proceed with the following procedure.

1. Turn the Dimension-P Raman off (i.e., turn the power switch on the back panel of the unit to “0” position).
2. Unplug the power cord of the Dimension-P Raman unit.
3. Open the fuse compartment using a flat screw driver or a similar tool.
4. Replace the fuse(s) and close the fuse compartment.

Send Unit Back to Manufacture For Repairs

Upon any other hardware failure, including:

1. The fan does not function as indicated by the fact that there is no air circulation near the fan vent at the back panel of the instrument.
2. The laser does not function as indicated by a zero power reading no matter how you adjust the laser power through the laser adjustment sliding bar (see Chapter 5, section “Laser Setting”->”Power Adjustment” for more details).
3. The CCD camera does not function as indicated by the fact that
    a. The CCD cooling temperature cannot reach the target temperature (-70 °C for Dimension-P1 Raman and -15 °C for Dimension-P2 Raman);
    b. No signal of the sealed cyclohexane standard sample can be acquired. To make sure that this is indeed a CCD camera failure instead of a communication failure between

the CCD and RamanSoft, check to make sure that the USB 2.0 cable is plugged both to the USB 2.0 port of the Dimension-P Raman unit and the USB port of your computer. If the problem persists, proceed to follow the directions in Chapter 5, section “RamanSoft Troubleshooting”->”CCD Camera Communication Failure”. A yet still persisting problem indicates that the CCD camera does not function.

You may need to send the unit back to manufacture for repairs. Before sending any product or parts for service or repairs, please contact Lambda Solutions, Inc. or your authorized representative or distributor (see Warranty & Service at the end of this manual for more details).

3 Installing RamanSoft

RamanSoft and PVCAM® Installation

RamanSoft must be installed under Windows. It can not be installed on a computer only running DOS, or from a DOS shell.

RamanSoft Installation is normally provided on a CD that contains a single setup executable file (Setup,exe). By double clicking on the Setup.exe or by typing in Setup.exe in the Run command window, RamanSoft InstallShield will start the installation process and will run until the completion of the installation.

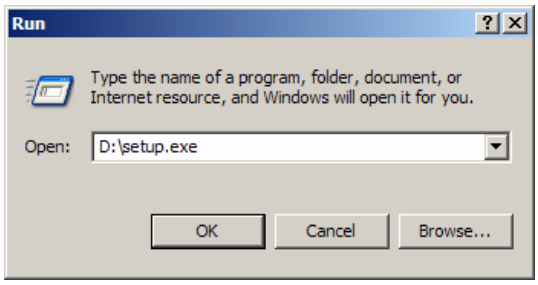
Note: When RamanSoft is installed, it modifies the Windows Registry file. If for any reason, a user reinstalls Windows, the Registry file may be replaced, and RamanSoft may not run correctly. Reinstall RamanSoft to correct this problem.

Note: If Installing under Windows XP, 2000, and NT, the user must be logged on as administrator. Certain changes are made to the Registry during the installation. If the user didn't log in as administrator, the Registry changes cannot be made and installation will fail.

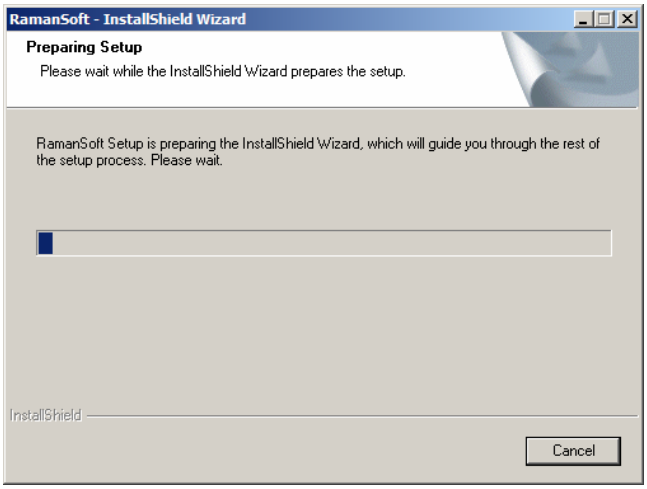
Take the following steps to install RamanSoft onto your computer.

- Exit any software currently running.
- Insert the RamanSoft Installation CD into your CD drive. Windows will detect the CD and the installation will begin automatically.

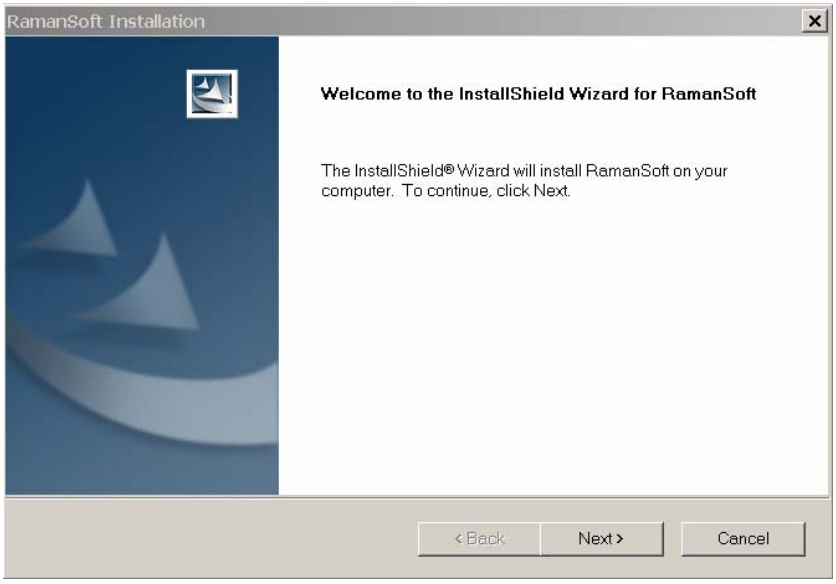
Note: If the auto-start feature has been disabled on your computer, click the desktop Start menu, select Run, type in or browse to D:\Setup.exe (where "D" is the letter designating your CD drive, as shown below) and press *OK*, the installation will begin.



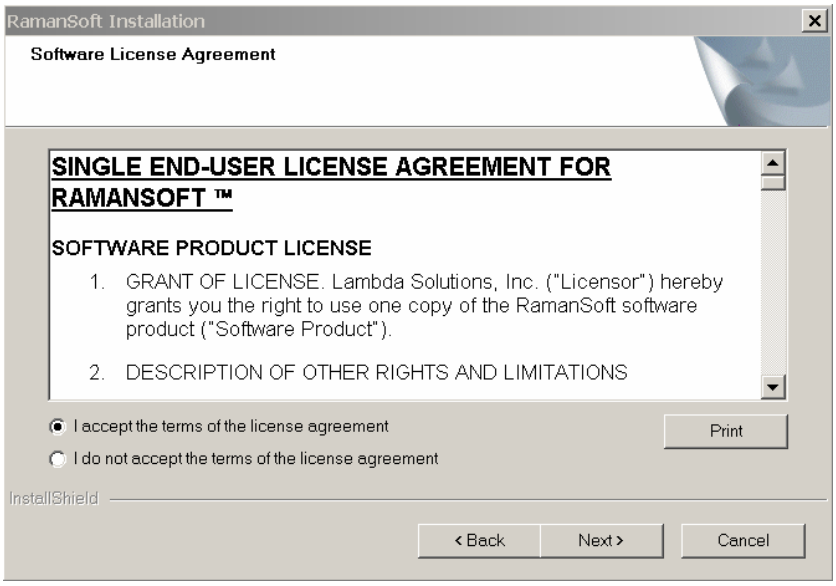
The Preparing Setup screen will appear indicating that the InstallShield setup program is loading.



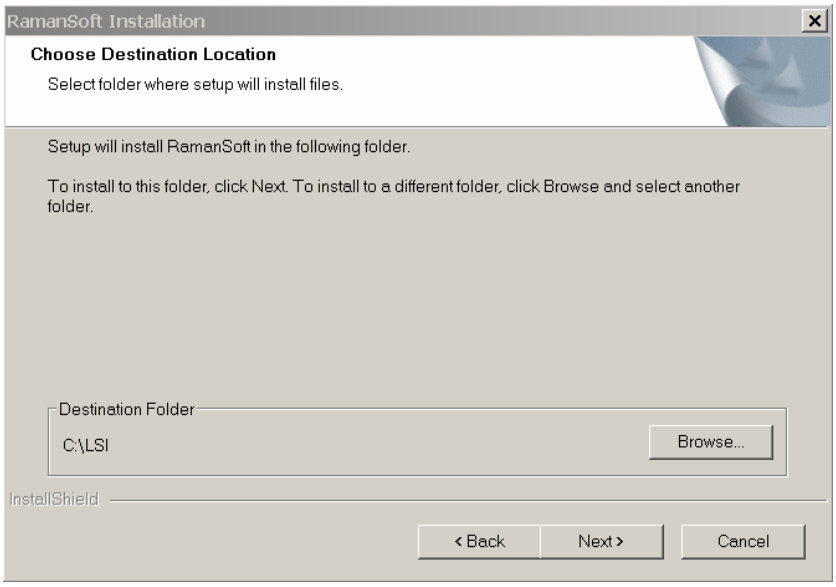
- The RamanSoft Installation welcome screen will appear as shown below. To quit without installing the software, click *Cancel*. To continue installing the RamanSoft, click on *Next*.



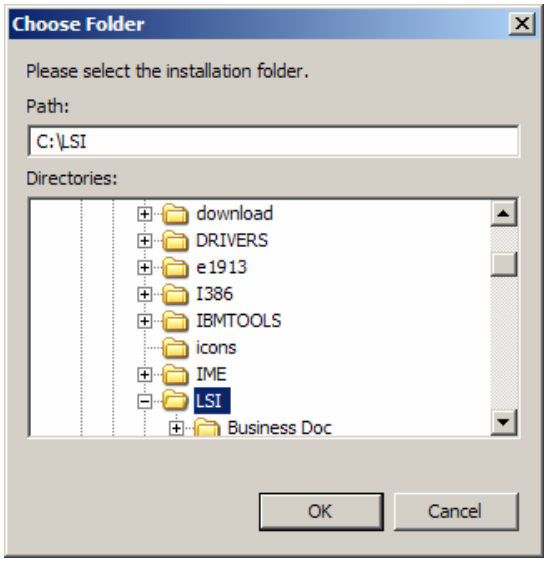
- Click *Next* to continue. The Software License Agreement screen appears next. Click *Yes* to continue. Click *No* to abort the installation or press *Back* to return to the previous screen.



- Next the Choose Destination Location screen appears. This dialog box allows the user to specify the directory where RamanSoft files are to be installed. A default location, C:\LSI, is recommended.

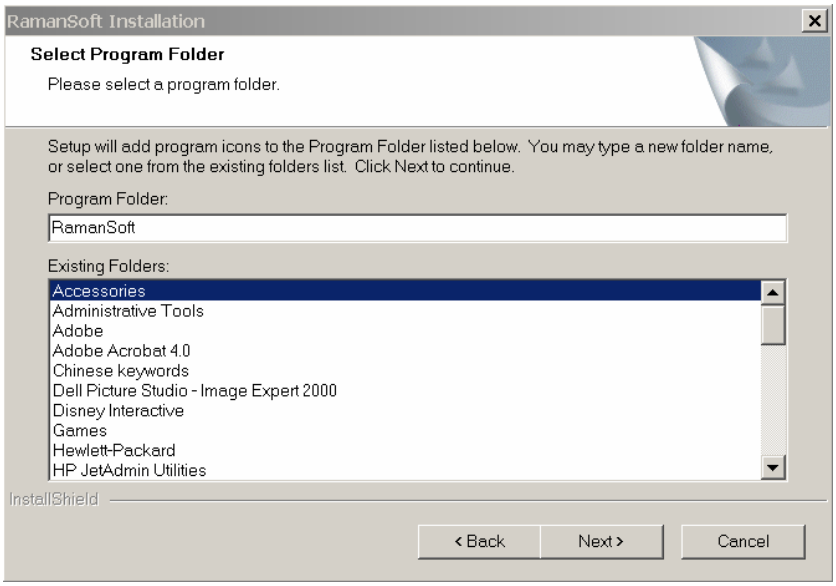


- To specify a different location, click on *Browse*. Then either select new directory or enter the path and name in the entry box and click *OK*.

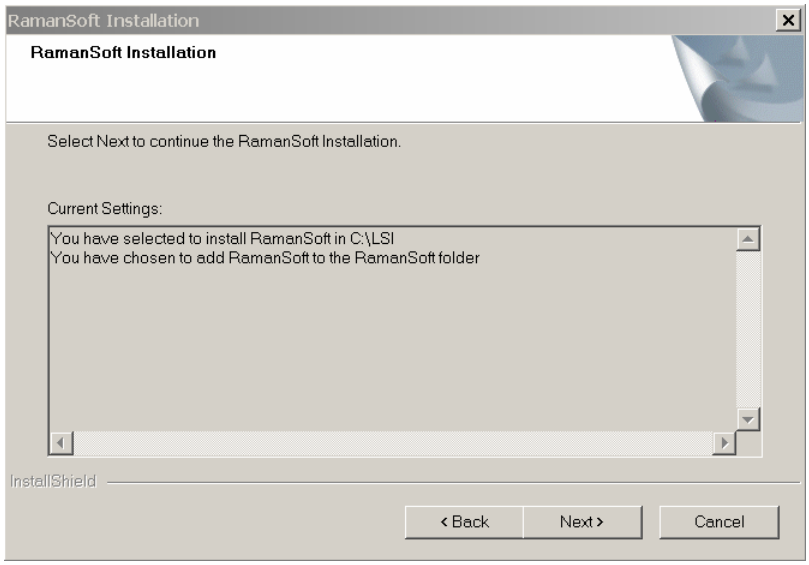


- Click on *Next* to bring up the Select Program Folder screen. Select either an existing folder or type in the name of a new folder.

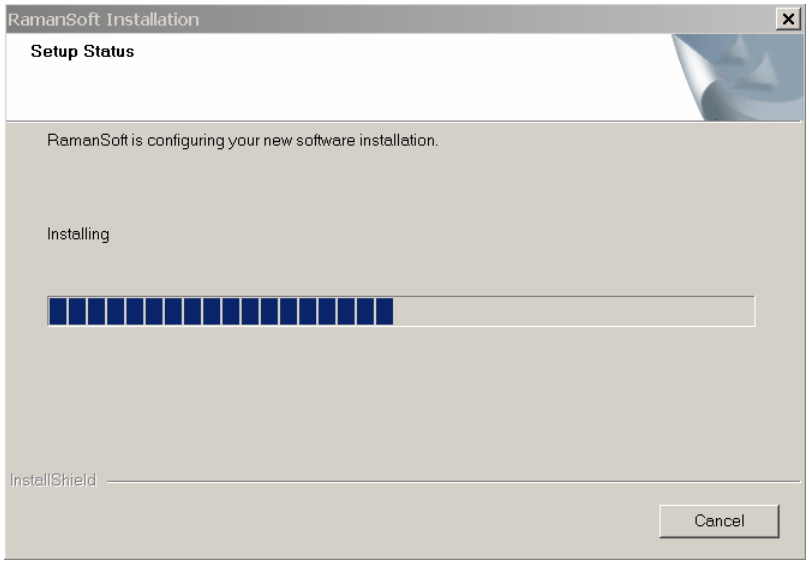
> **Note**: This is not the folder where the files are to be installed, but rather the *Start* menu folder from which RamanSoft can be launched.



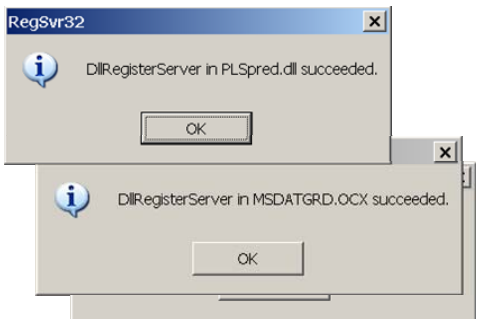
- The current folder setting screen will appear next (shown below). It lists the destination folders. Click on *Next* to initiate the actual installation in which the files will be decompressed and placed in the specified directory. Click on *Back* to make any changes or click on *Cancel* to exit the procedure.



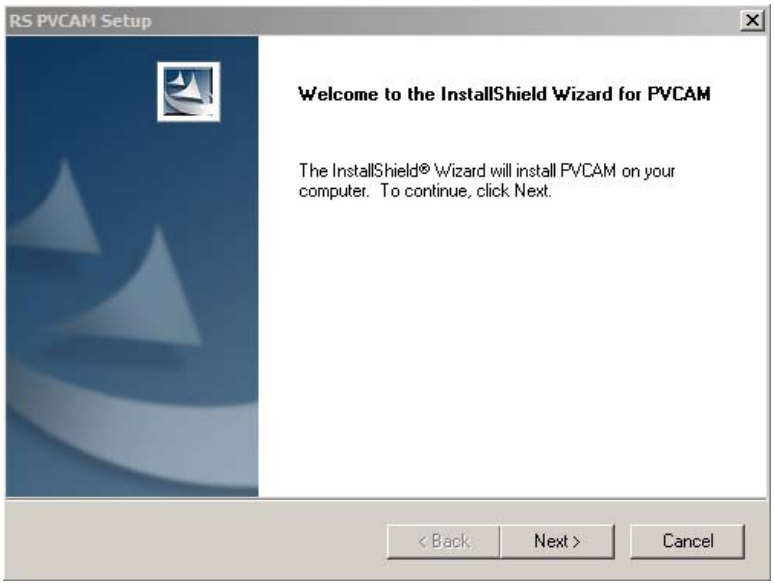
- The Installation Setup Status screen (shown below) appears next. The names of the individual files appear in the message box as they are loaded and the installation progress in percent is continuously displayed.



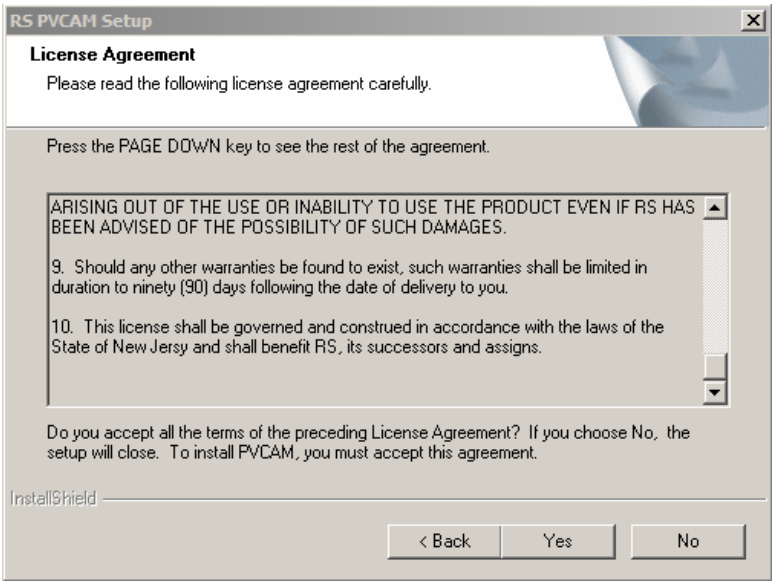
- After file installation, the RamanSoft InstallShield will start to register the DLLs which RamanSoft has incorporated into the Windows' Registry. The following message box will appear after the Windows' registration is succeeded and finished.



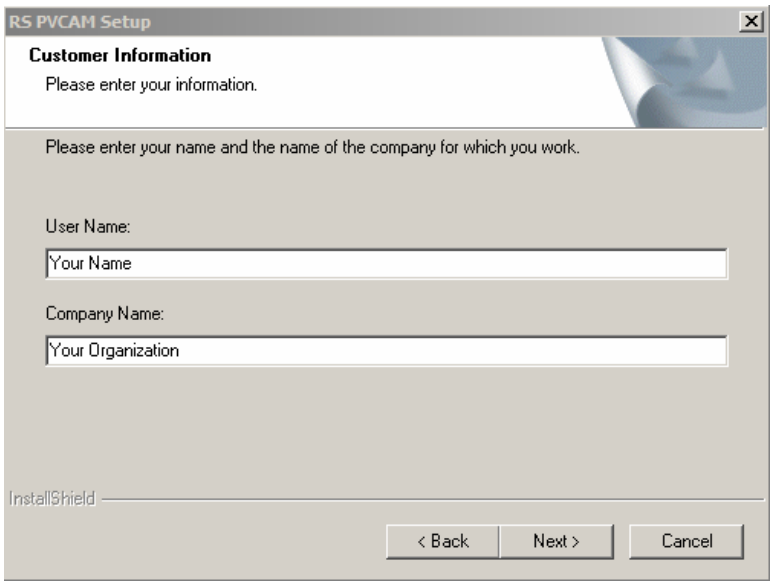
- Next, the InstallShield will automatically start the installation of Princeton Instruments' PVCAM® (Programmable Virtual Camera Access Method), a library that can be used to control and acquire data. The following Setup window appears.



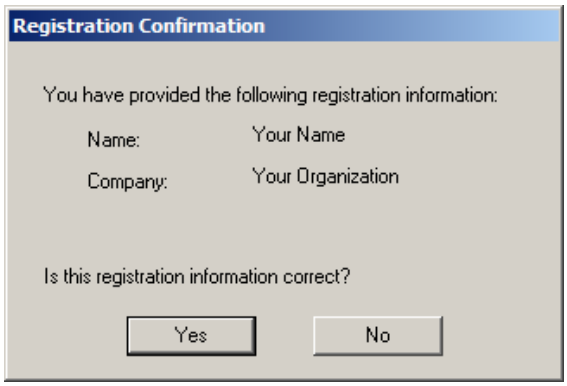
- Click on *Next*, PVCAM License Agreement Window appears. Click on *Yes* to accept the agreement and continue the installation.



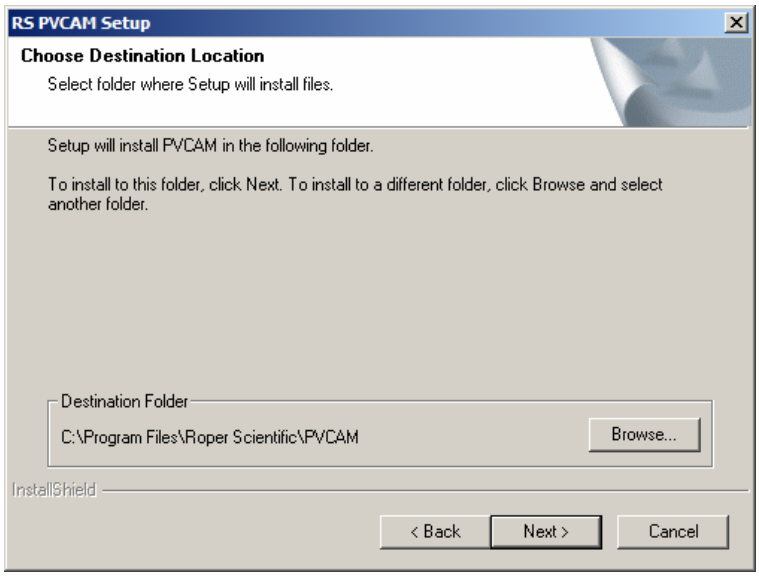
Next, the Customer Information dialog box appears. To continue, enter your name and organization. Then click *Next*.



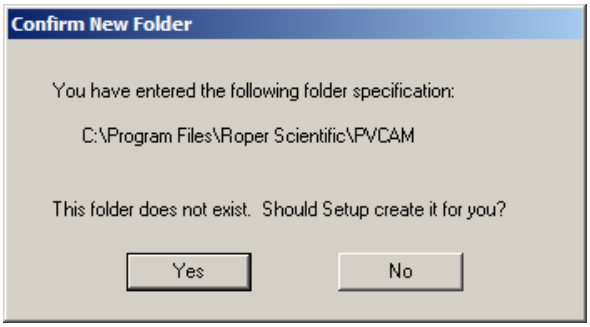
- You are next asked to confirm your registration information via the following dialog box. Select *Yes* if the displayed information is correct and you want to continue. Click *No* to return to the Customer Information window to make necessary changes.



- Next, the Choose Destination Location dialog box appears. This dialog box allows you to specify the directory where you would like the PVCAM files to be installed. A default location, C:\Program Files\Roper Scientific\PVCAM, is suggested.

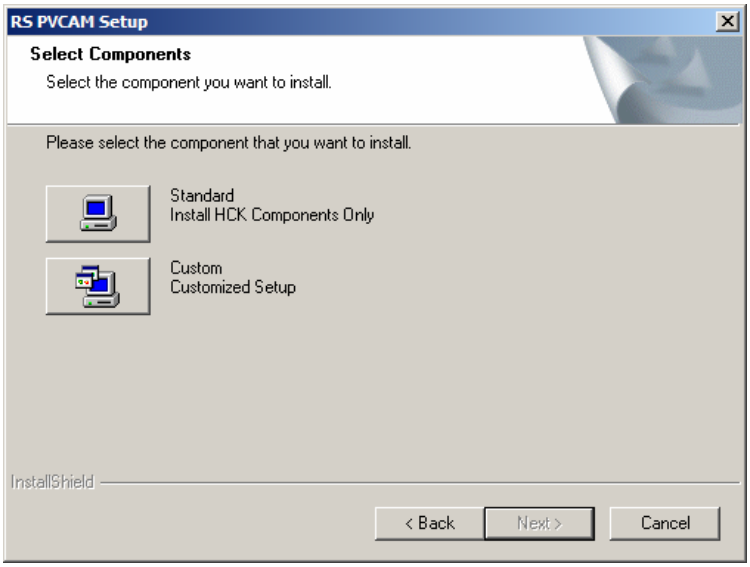


- To specify a different location, click on *Browse*. Then either select a new directory or enter its name (with path information) in the entry box and click *OK*. If the name of the directory does not exist, the installation will offer to create it for you.



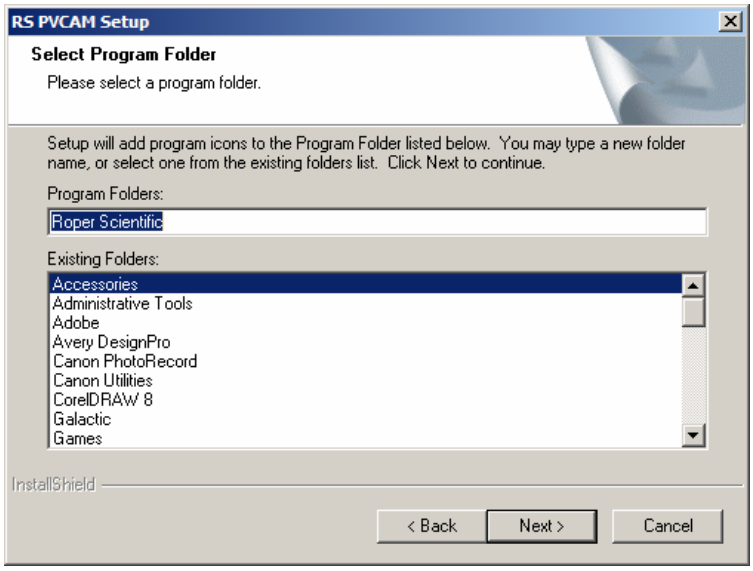
- Next, the Setup Type dialog box appears. You are given the following choices:

  Standard: Install DLLs and PCI interface driver.
  Custom: Allows user to choose files to be installed.

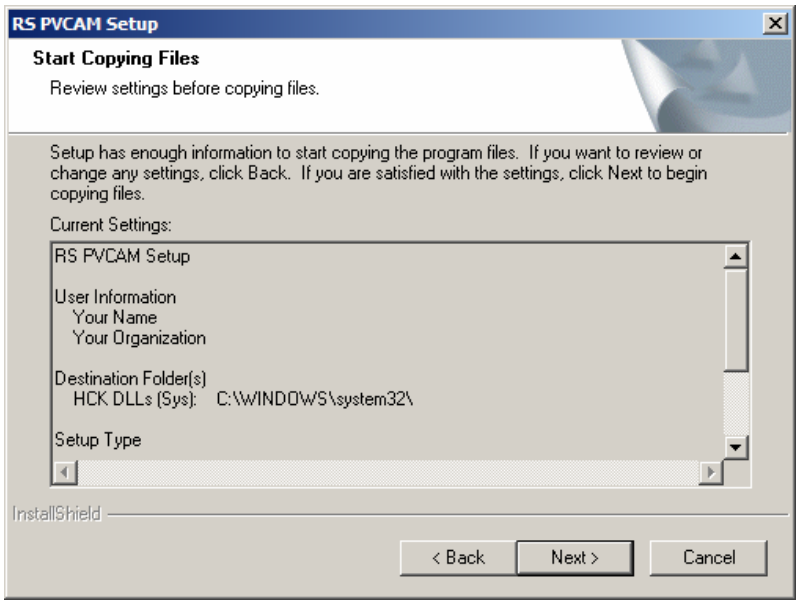


- Choose either *Standard* or *Custom* setup and click on *Next* to bring up the Select Program Folder dialog box. You can either select an existing folder or type in the name of a new one.

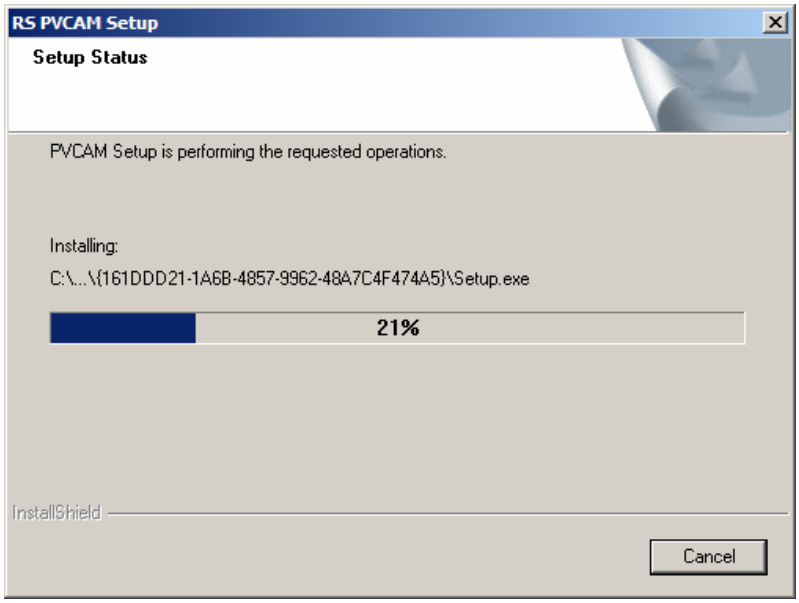
> **Note**: This is not the folder where the files are to be installed, but rather the Start Menu folder from which RamanSoft can be launched.



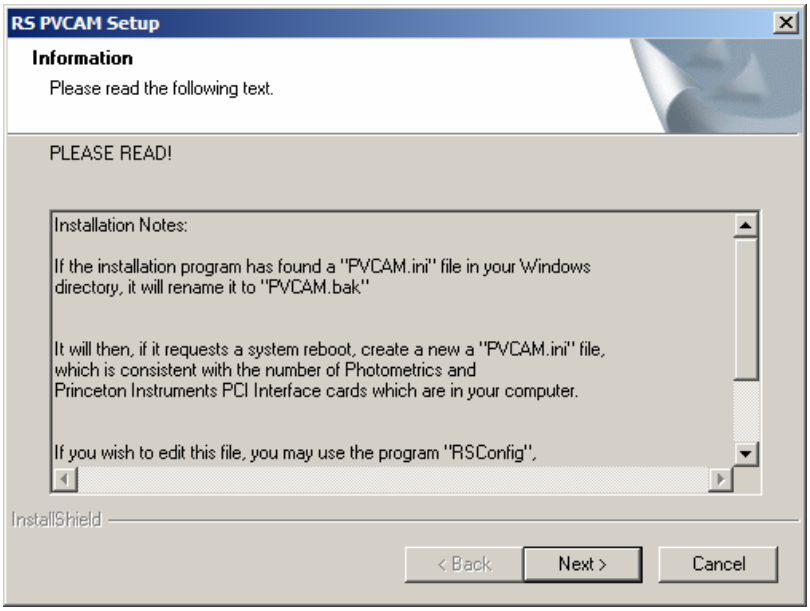
- The Start Copying Files dialog box appears next. It lists User information, the Destination Folder(s), the Setup Type and the Program Folder. Click on *Next* to initiate the actual installation in which the files will be decompressed and placed in the specified directory. Click on *Back* if the user wishes to make any changes, or click on *Cancel* to exit the procedure.



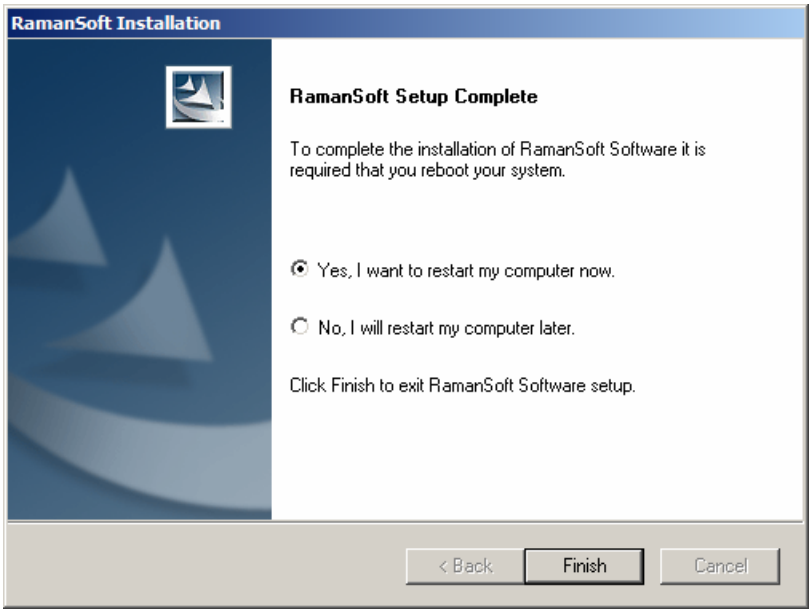
- The Setup Status screen (shown below) appears next. The names of the individual files appear in the message box as they are loaded and the installation progress in percent is continuously displayed.



- After the files are installed, an information dialog about PVCAM.ini file location and RSConfig program appears.



- Finally, the RamanSoft Setup Complete dialog appears and asks you to restart the computer now or later. Before you can use the newly installed the RamanSoft, the computer has to be restarted.

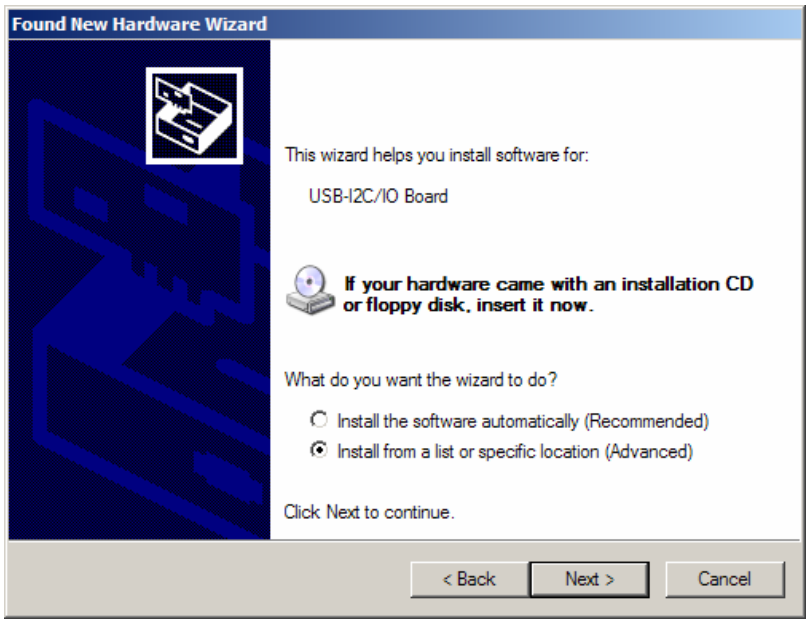


- Click on *Finish* to restart the computer and finish the RamanSoft installation.

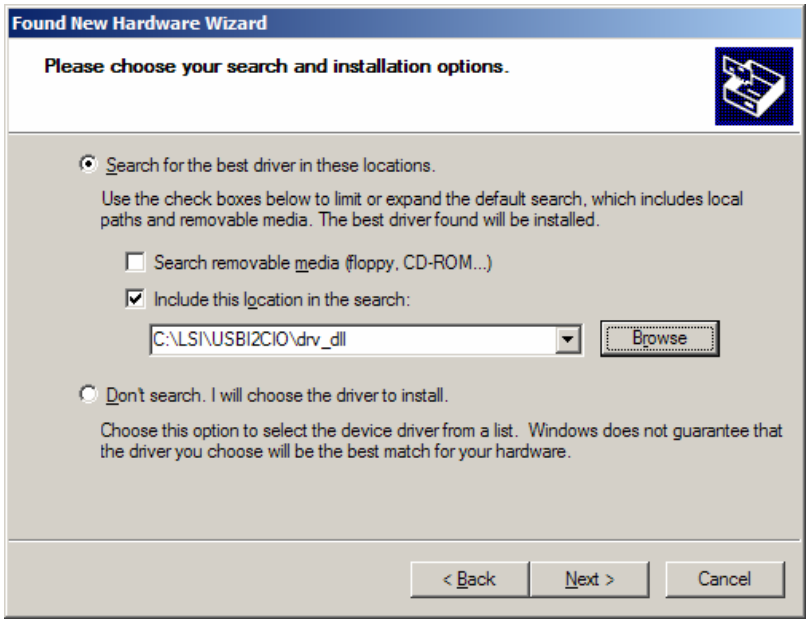
Setting up the LSI Laser control board USB 2.0 Interface

Administrator privileges are required under Windows 2000 and Windows XP to install the RamanSoft interface control for the Dimension Raman System's hardware.

Once the user finished the RamanSoft Installation, and when the USB 2.0 cable is connected to the computer for the **first** time, the following Found New Hardware Wizard window will appear.



For the first time, the user should choose *Install from a list or specific location* as shown above. Then click *Next*.



On the window shown above, the user should choose *Search for the best driver in these locations* and browse to C:\LSI\USBI2IO\drv_dll folder as shown above. Click on *Next*

Default Directories

A full installation of RamanSoft will create directories and copy files as listed below. The local drive is assumed to be C:.

C:\LSI\RamanSoft

This directory contains following folders:

- The **Calibration** folder stores the calibration files whenever a calibration is performed on the system.
- The **Configuration** folder contains the configuration files which store file settings, configuration parameters, and system settings that user creates. A default configuration file is created when RamanSoft is installed.
- The **Dll** folder contains all the DLL files required to run RamanSoft that are installed into windows system file.
- The **Exe** folder contains all the executable RamanSoft files. There are three of them. RamanSoft.exe is the file that is run when user clicks on the RamanSoft icon. SpectrumPredict.exe and SpectrumSearch.exe are macro executable files ran by RamanSoft to perform spectrum search and spectrum predict.
- The **Init** folder contains all the system init files that RamanSoft requires. These files are also installed in Windows directory. The files in the init folder serve as a backup in case these important system init files were corrupted. In this case the user can copy these files back to the Windows directory.
- The **Log** folder contains all the log files that the system registers for every active RamanSoft session.

C:\LSI\RamanData

This is the Default directory for data files. This directory contains following folders:

- The **CalibrationData** folder contains all the calibration spectrum data when a system calibration is performed.
- The **SystemTest** folder contains all the system test spectrum data when a system test is performed.

C:\LSI\ USBI2CIO

This directory contains all drivers and dlls required for LSI Laser Control Board USB Interface. It contains the following folders:

- The **drv_dll** folder contains driver and dlls.
- The **Firmware** folder contains the firmware of LSI USB Laser Control Board.
- The **Utils** folder contains the recovery system files.

C:\LSI\ RunIQPredict

This directory contains all sample .Cal files and .iqs files for running SpectrumPredict in RamanSoft.

4 A Quick Tour

This chapter gives a quick tour on how to use the Dimension-P Raman system with the operating software RamanSoft. The purpose of this chapter is to familiarize the user with the tools for spectrum acquisition, spectrum processing and analysis. In addition, this Tour introduces and demonstrates the use of spectral library search, qualitative and quantitative spectral analysis, and real time process monitoring. Please refer to the reference chapters (Chap. 5 – 8) for a comprehensive description of the RamanSoft functionality.

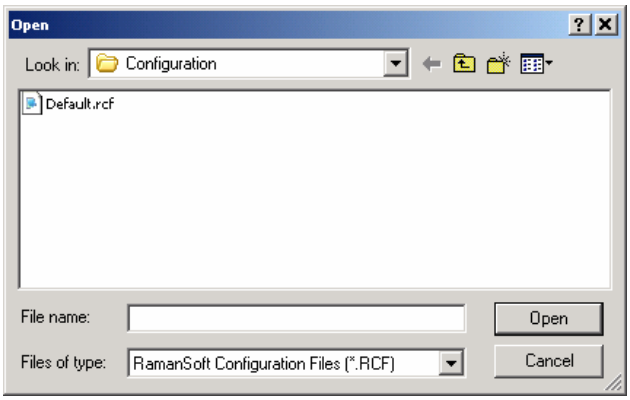
Throughout this chapter, we refer to the sealed cyclohexane standard sample that comes with the instrument for illustrative purposes. The Tour is demonstrated with Dimension-P1 HR Raman System, and the data are stored in C:\LSI\RamanData\Tour and they come with the system.

Starting Dimension-P Raman

- Turn on the power switch of the Dimension-P Raman (on the back panel of the instrument).
- Turn the Laser Power key to **ON** position.
- Turn on the computer.

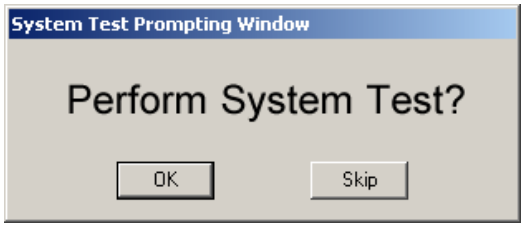
Starting RamanSoft

- From Windows® *start* menu, go to *All Programs* and choose *LSI->RamanSoft*. Or create a shortcut for RamanSoft from C:\LSI\RamanSoft\exe and place it on the desktop, double click the RamanSoft shortcut icon.
- Choose the default configuration file by clicking on the *Default.rcf* file icon, and click *Open*.

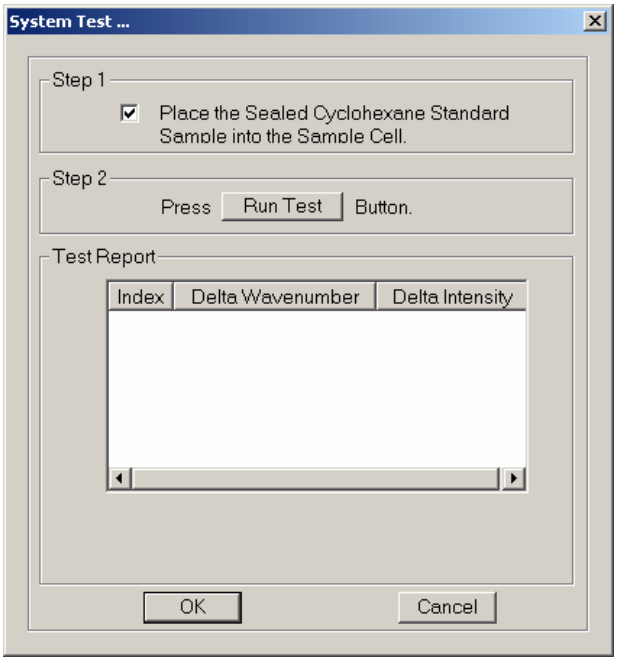


- Wait till the Laser Power Stabilizing Progress Window and the CCD Temperature Warning Window disappear (takes 15 seconds and 2-3 minutes, respectively).

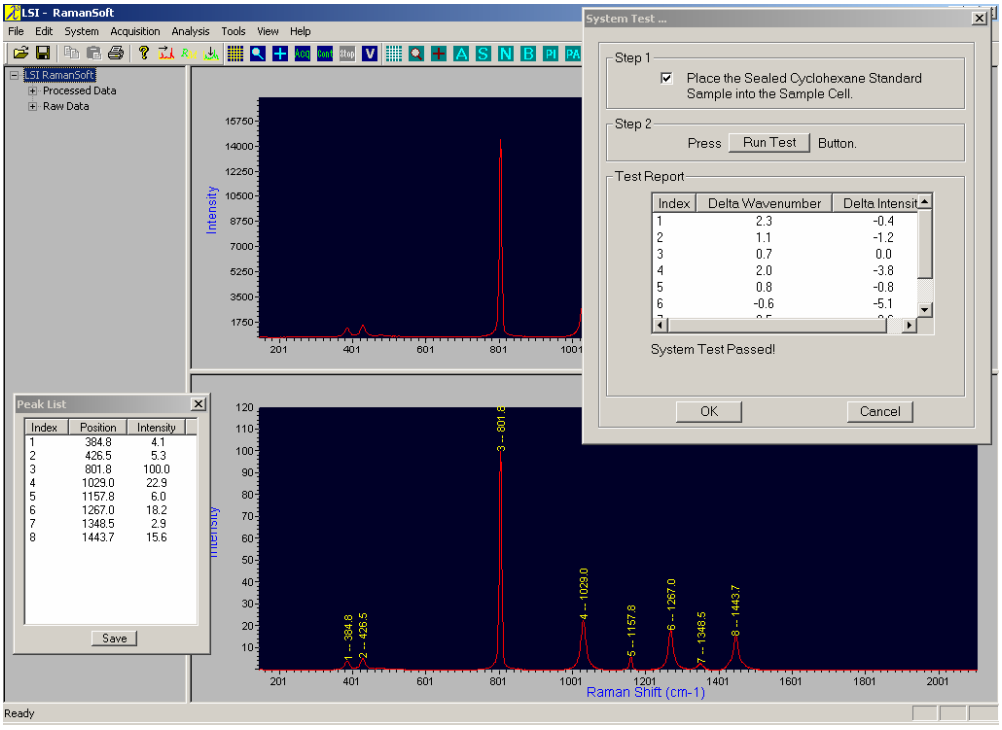
- Press *OK* on the System Test Prompting Window to perform a system test.



The System Test window outlines the steps for performing the system test. Check *Step 1*, and put the sealed cyclohexane standard sample in the internal sample cell or the External Sampling Module. If the External Sampling Module is used, be sure to close the lid.

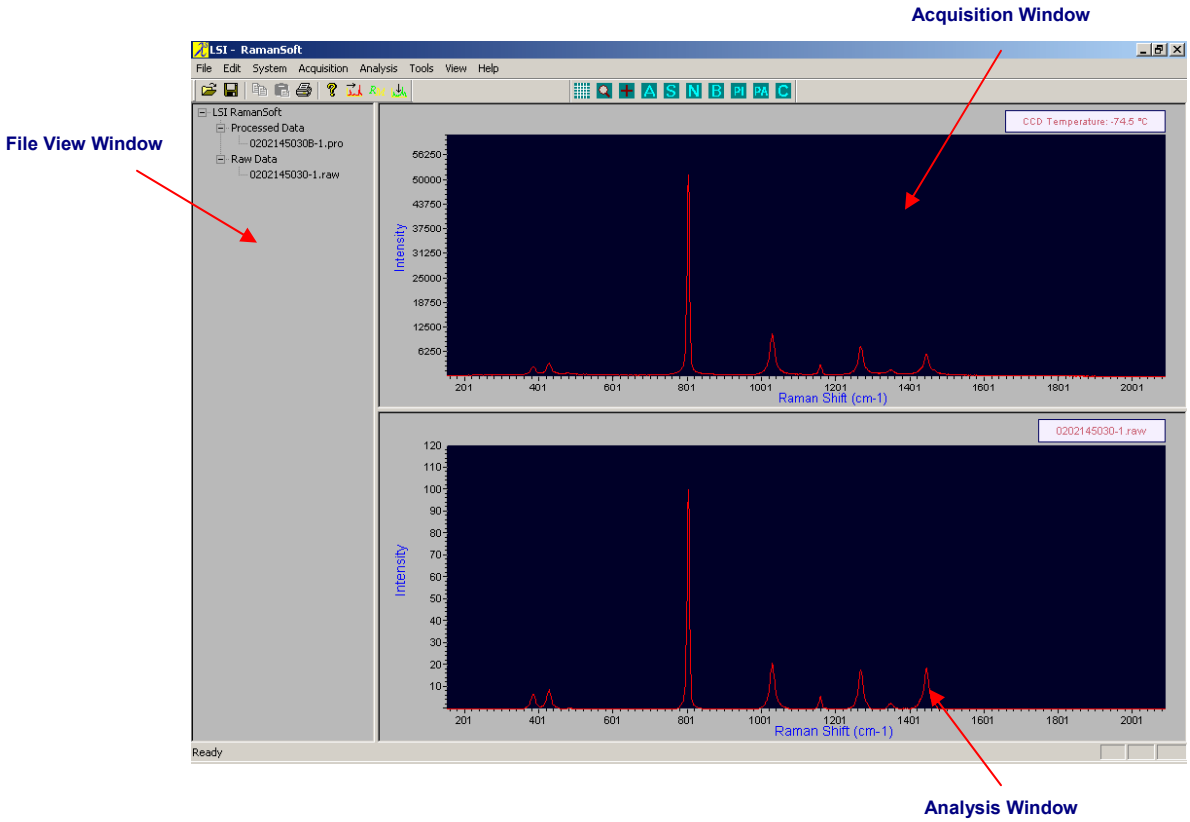


Press *Run Test*. The system starts to acquire the spectrum for the sealed cyclohexane standard sample, and the test results appear in the *Test Report* group box of the System Test widow.



If the system test is passed, click *OK* to continue the tour. If the test fails, a re-calibration of the instrument is then necessary. Please refer to section “Calibration” of Chapter 5, “RamanSoft Basic Functionalities”, to re-calibrate the system.

- The RamanSoft user interface appears as follows:



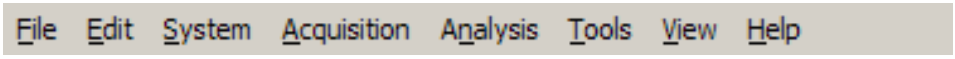
The Acquisition Window displays the acquired raw spectrum (of cyclohexane), the Analysis Window displays the processed spectrum (here the background-removed and normalized cyclohexane spectrum – see later sections on RamanSoft Data Processing methods), and the File View Window displays *Raw Data* files and the *Processed Data* files for the current RamanSoft session. Double clicking any of these files places that raw or processed spectrum in the Analysis Window.

Acquiring a Spectrum

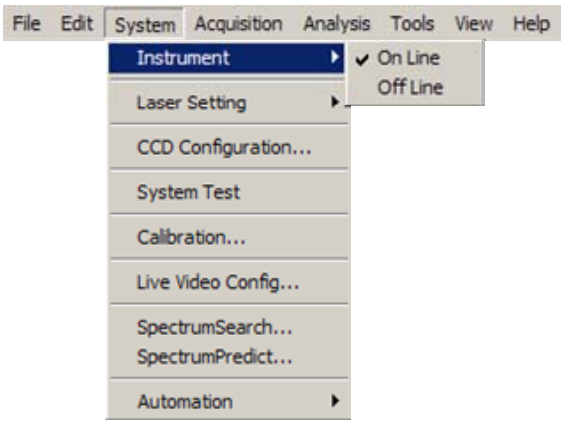
Before a spectrum is acquired, the user needs to set up acquisition parameters which include setting up the laser power, the CCD integration time, the number of frames to average in one measurement, the file name and directory etc. (Note that for the system test as shown above, these parameters are automatically set by the system).

Setting Laser Power

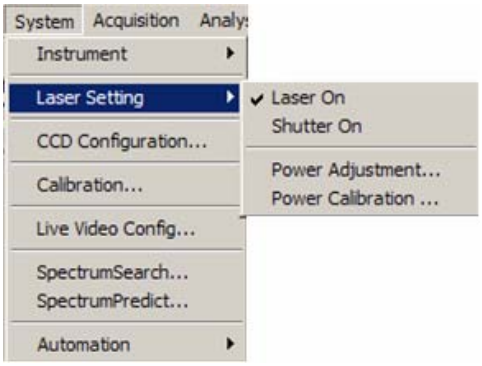
- From the main System menu of RamanSoft,



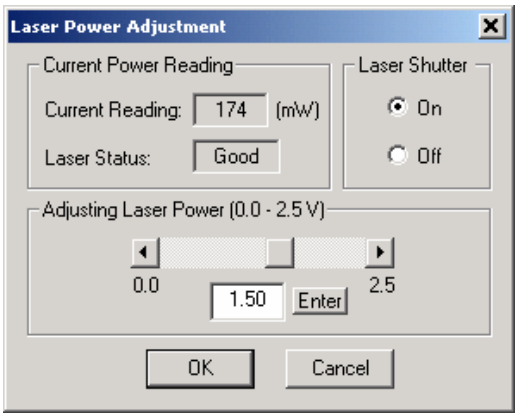
- choose *System->Instrument*. Make sure that *On Line* is checked.



- Go to *Laser Setting*, make sure that *Laser On* is checked.



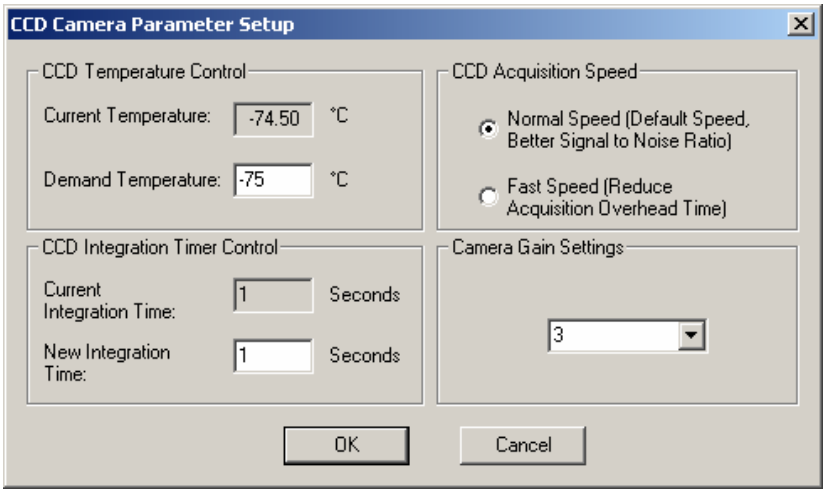
- Go to *Power Adjustment*…, the Laser Power Adjustment window appears:



Set the laser power to the desired value (100 – 200 mW is recommended) by dragging the bar in the *Adjusting Laser Power* (*0.0 – 2.5 V*) group box, or by entering a number between 0 and 2.5 in the text entering box. The adjusted laser power is displayed in the *Current Reading* of *Current Power Reading* group box. Click *OK*.

Setting CCD Integration Time

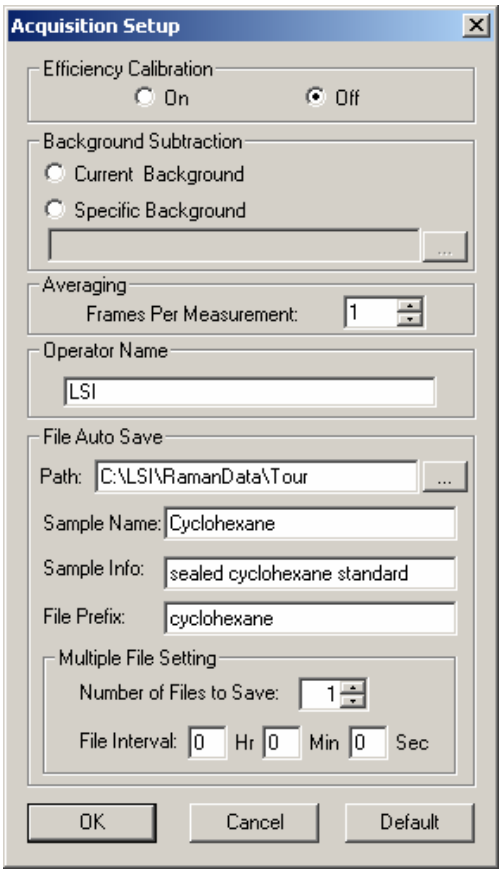
- From *System*, choose *CCD Configuration*…, the CCD Camera Parameter Setup window appears:



Set the *New Integration Time* to be what is desired (1 second is usually sufficient and recommended for most solid and solvent samples, longer integration time is recommended for aqueous samples). Signal intensity may also be adjusted with the Gain Setting, with 3 yielding the highest signal. Leave CCD Acquisition Speed as *Normal Speed* (default).

Setting Acquisition Parameters

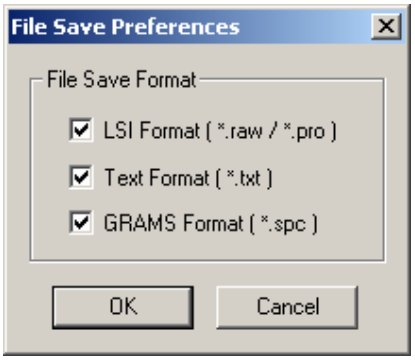
- From the main menu, choose *Acquisition->Setup*, the Acquisition Setup window appears:



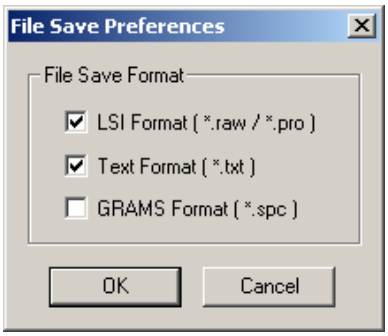
The *Frames Per Measurement* in the *Averaging* group box can be left as default (1, i.e., no averaging). Type in appropriate information in the text boxes for *Operator Name*, *Path* (the name of the folder set up within the RamanData folder), *Sample Name*, *Sample Info*, and *File Prefix*. Note that by installing RamanSoft, you have already have the directories C:\LSI\RamanData created on your computer. For your tour purpose, you are recommended to create a subfolder \Tour2 to store your actual tour data so as not to overwrite the sample tour data in C:\LSI\RamanData\Tour that comes with your system. To do this, type in "C:\ :\LSI\RamanData\Tour2" in the *Path* field and the program will automatically create the subfolder "\Tour2" within \RamanData.

Setting File Preferences

- From the main menu, choose *File->Preferences*, the File Save Preferences window appears:



By default, each acquired spectrum is saved in *LSI Format* (**.raw* for raw spectrum, **.pro* for processed spectrum), *Text Format (*.txt*), and GRAMS Format (*.spc) so as to conveniently import data to Thermo Electron Corporation's GRAMS software suite such as GRAMS/AI™. For this tour, we'll only save the data in the default LSI Formats and the Text Format by deselecting the checkbox for GRAMS Format (*.spc).

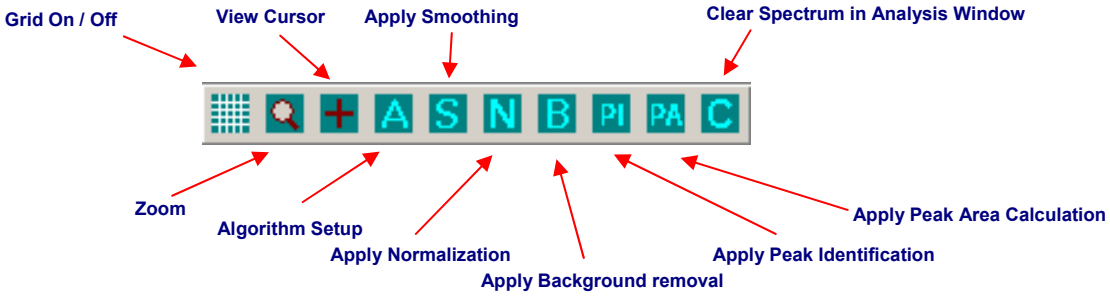


Acquiring a Spectrum

Before acquiring a spectrum and saving it into a file, it is helpful to view the spectrum under current acquisition settings and probe working distance first, and if desired, the acquisition settings can be adjusted according to the quality of the spectrum. This can be done through the *Continuous Acquire* function of RamanSoft.

Getting Ready

- Put the sealed cyclohexane standard that comes with the instrument into the internal sample cell or the External Sampling Module. If the External Sampling Module is used, be sure to close the lid.
- Click anywhere within the Analysis Window, the Analysis Window Toolbar appears in the system toolbar panel, which is below the main menu items but above the Acquisition Window:



Click on  to clear the spectrum in the Analysis Window from the system test.

Continuous Acquiring

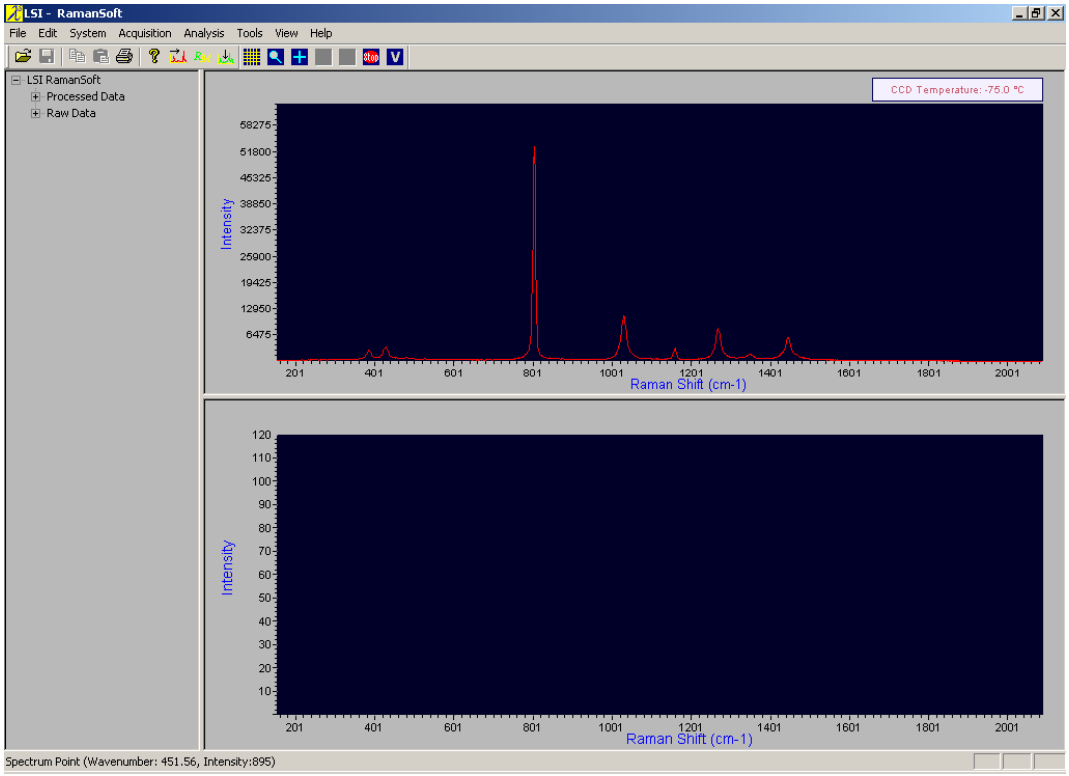
- From the main menu, choose *Acquisition*, the Acquisition submenu appears.

- Click *Continuous Acquire*. Alternativley, the continuous acquire function can easily be invoked by pressing Cont on the Acquisition Window Toolbar (click anywhere in the Acquisition Window to bring up the Acquisition Window Toolbar).

- In case the Laser Power Status Warning Window appears as below,

click *Yes*. Wait till the Laser Power Stabilizing Window disappears. Note that this window sometimes appears as the system automatically turns off the laser if the system has been idle for more than 30 minutes.

- If you have gone through the above step to turn on the laser, you need to click on *Acquire* -> *Continuous Acquire* or press Cont again. The program first acquires a dark current and subsequently the Cyclohexane spectrum is acquired and displayed in the Acquisition Window, and it is updated every 1 second.



Note that in the continuous acquisition mode, the Stop button on the Acquisition Window Toolbar becomes active. Click *Acquisition -> Stop Acquire* or press Stop to exit the continuous acquisition mode.

Acquiring a Spectrum

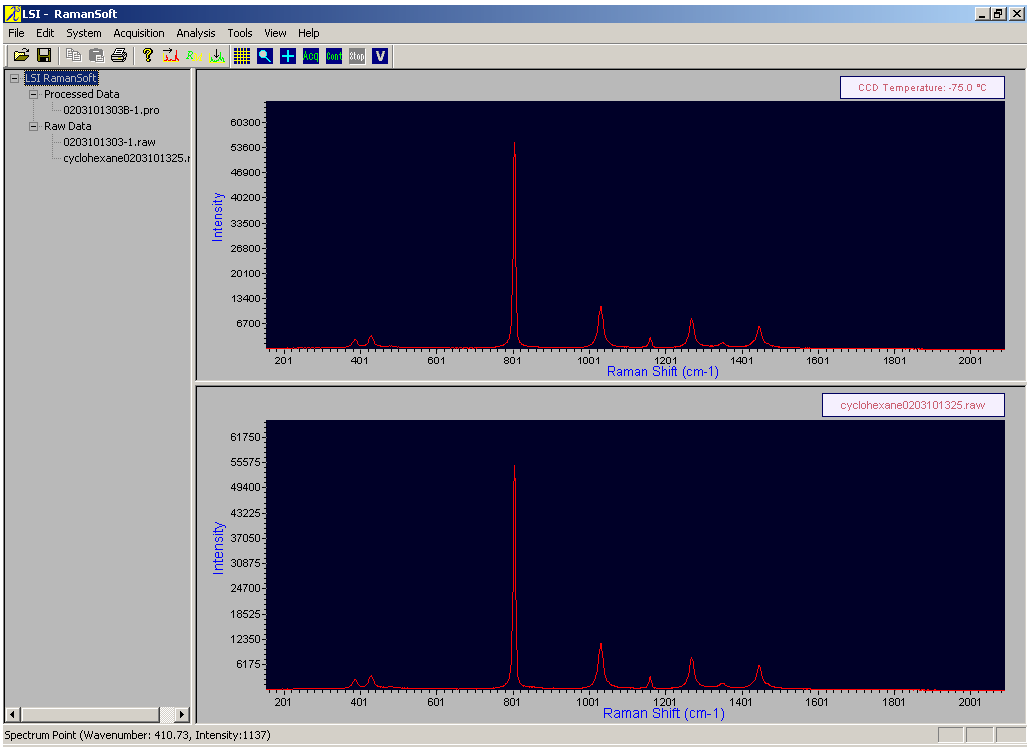
- To acquire a spectrum, click *Acquisition -> Acquire* or press Acq on the Acquisition Window Toolbar, the spectrum is displayed in the Acquisition Window.

Saving a Spectrum

A spectrum can be saved using either a system-generated file name or a user-specified file name.

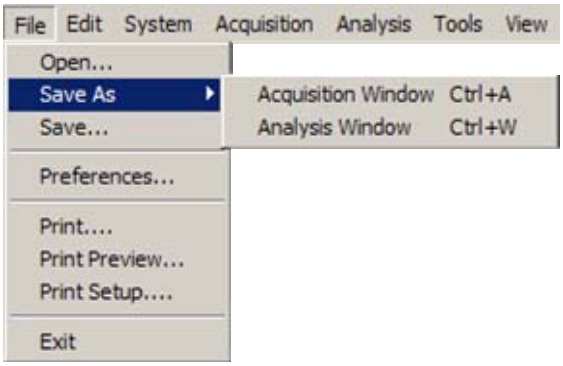
Using a system-generated file name

- From the main menu, choose *File->Save*…, the spectrum is then saved in the folder specified by the *Path* field, using the system generated filename, i.e., the *File Prefix* that the user has put in the Acquisition Setup window (see section “Set Acquisition Parameters”) followed by a computer generated date and time string. For example, the file for this tour is saved as C:\LSI\RamanData\Tour\cyclohexane0203101325.raw. Note that the saved spectrum is displayed in the Analysis Window and the filename is added onto the “Raw Data” list in the File View Window.

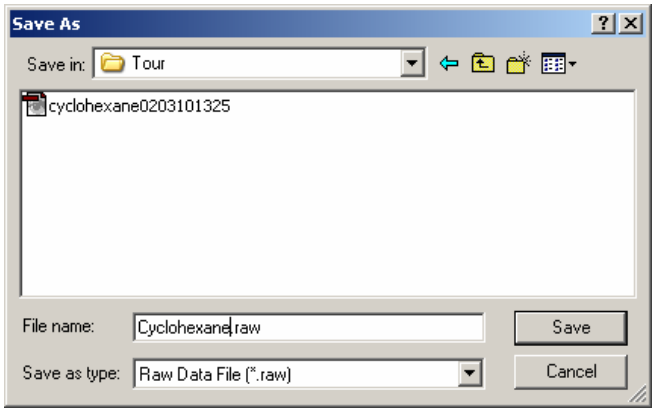


Using a user-specified file name

- Alternatively, the user can save the acquired spectrum using a user-specified filename. From the main menu, click *File->Save As->Acquisition Window*:

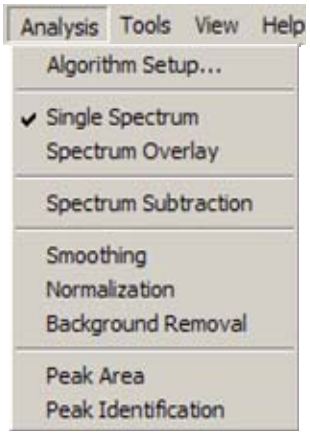


Choose a desired directory, and input the file name. The file extension ".raw" (stands for "raw data") is already there. Replace the "*" with the user-specified file name. For this tour, save it as "Cyclohexane.raw". Note that the filename is added onto the "Raw Data" list in the File View Window. (Note that your raw data file may not take the Adobe Photoshop file icon as in the figure below. See section "Save As" in Chapter 5 for an explanation).



Spectrum Processing / Analysis

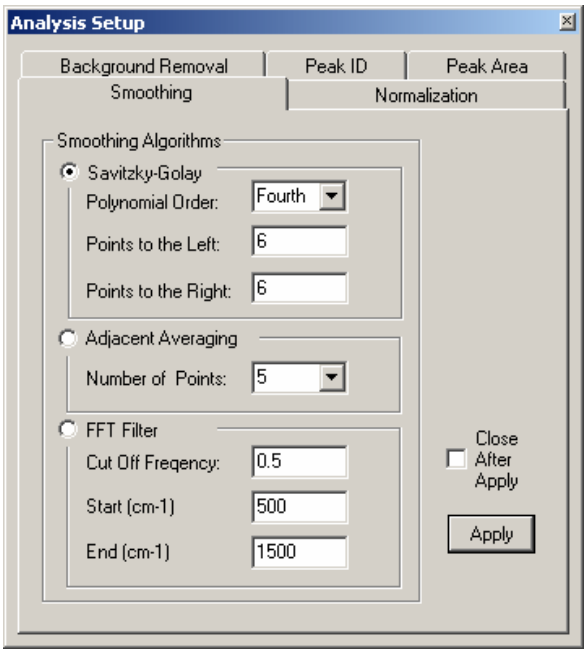
To perform data processing/analysis (smoothing, normalization, background removal, peak identification, peak area calculation, and spectrum subtraction) on the acquired spectrum, click on Analysis from the main menu.



By default, a data processing/analysis method is applied to one spectrum at a time (note that *Single Spectrum* is checked by default). To set up a particular algorithm to use for each method, click *Algorithm Setup*…. The Analysis Setup window appears. Click on the page tab to bring up the algorithm setup page for a desired method.

Smoothing

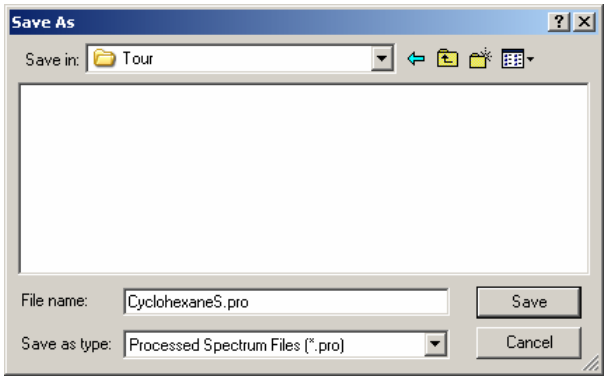
- Click on the *Smoothing* tab in the Analysis Setup window to bring up the Smoothing Algorithms page.



- Click on  in the Analysis Window Toolbar to clear the Analysis Window. In the File View Window, double click on the file name “Cyclohexane.raw” in the Raw Data list to bring the spectrum to the Analysis Window.

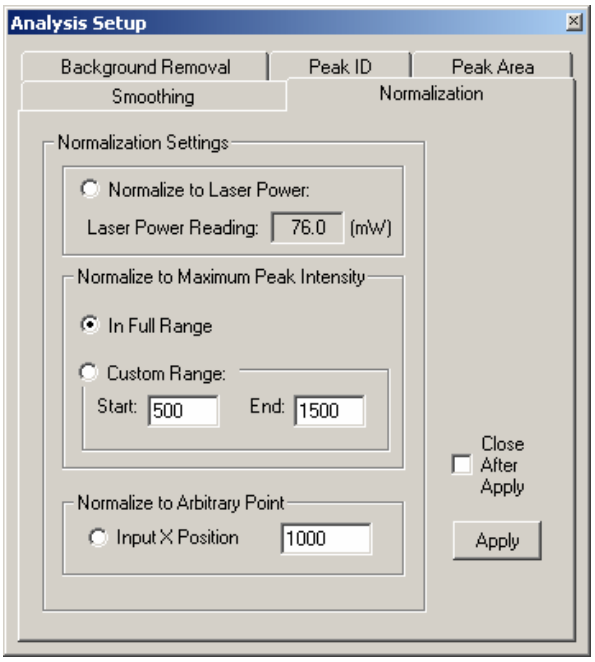
- Click *Apply* to apply the default Savitzky-Golay smoothing algorithm to the spectrum. The smoothed spectrum is now displayed in the Analysis Window.

- To save the smoothed spectrum, click *File->Save As->Analysis Window*:
  Save the smoothed spectrum as “CyclohexaneS.pro”. Note that this filename is added onto the “Processed Data” list in the File View Window.



Normalization

- Click on the *Normalization* tab to bring up the Normalization Settings page.



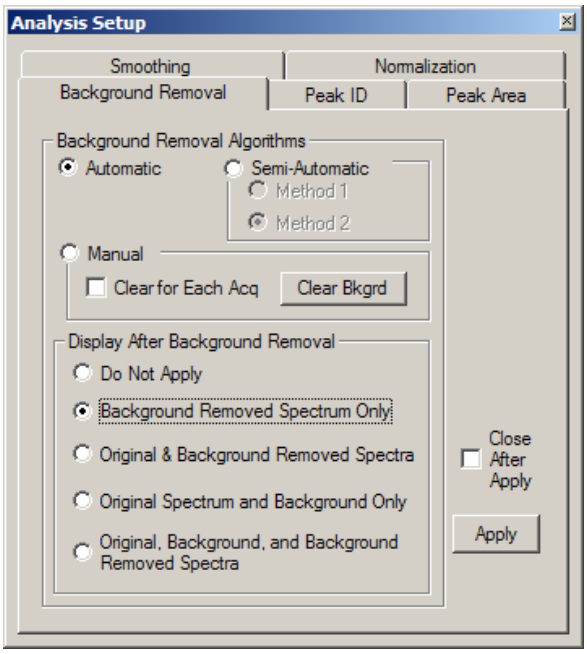
- Click on  in the Analysis Window Toolbar to clear the Analysis Window. In the File View Window, double click on the file name “Cyclohexane.raw” in the Raw Data list to bring the spectrum to the Analysis Window.

- Click *Apply* to apply the default *In Full Range* normalization algorithm to the spectrum, which normalizes the spectrum to the maximum peak intensity. The normalized spectrum is now displayed in the Analysis Window. Note that the maximum intensity is scaled to 100 (arbitrary units) for a normalized spectrum.

- To save the normalized spectrum, click *File->Save As->Analysis Window* and save the normalized spectrum as “CyclohexaneN.pro”.

Background Removal

- Click on the *Background Removal* tab to bring up the Background Removal Algorithms page.



- Click on  in the Analysis Window Toolbar to clear the Analysis Window. In the File View Window, double click on the file name “Cyclohexane.raw” in the Raw Data list to bring the spectrum to the Analysis Window.

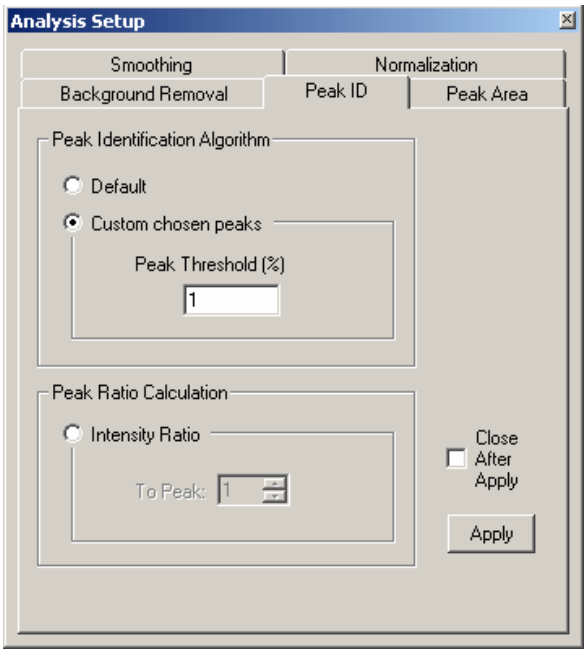
- Click *Apply* to apply the default *Automatic* algorithm to the spectrum, which automatically removes the background from the raw spectrum. The background-removed spectrum is also efficiency-corrected, and is now displayed in the Analysis Window. Note that the “baseline” of the spectrum is more flatly aligned up with the x-axis.

- To save the normalized spectrum, click *File->Save As->Analysis Window* and save the background-removed spectrum as “CyclohexaneB.pro”.

Peak Identification / Intensity Ratio

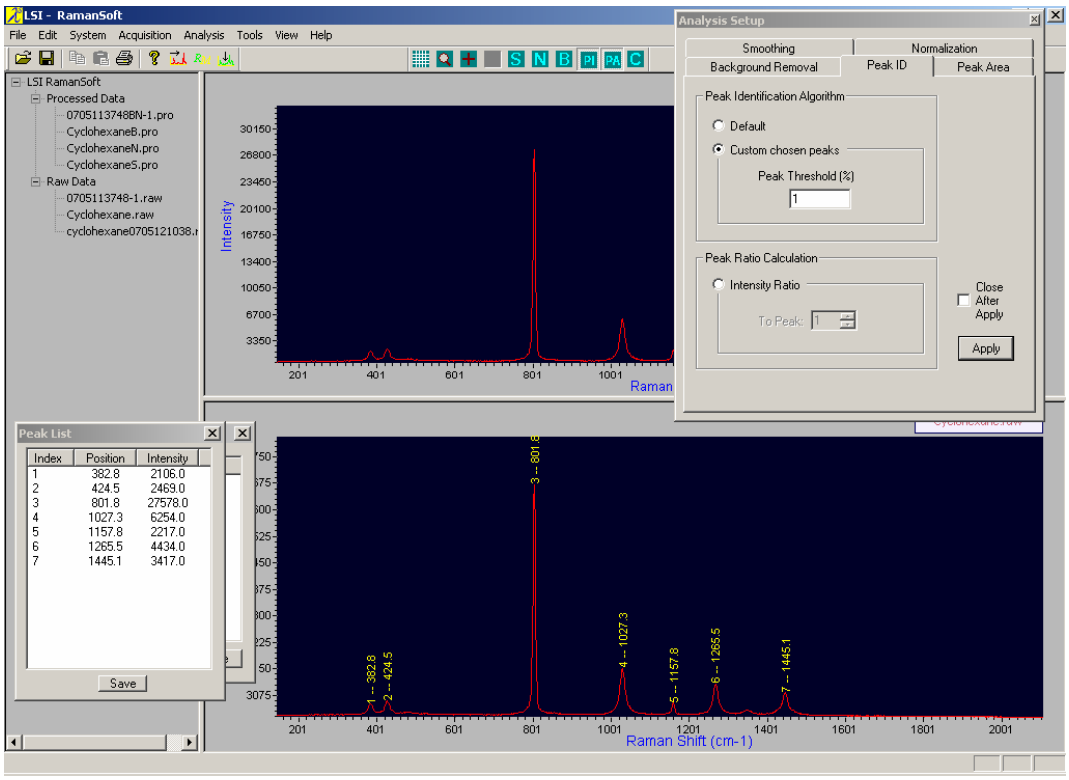
- Click on the *Peak ID* tab to bring up the Peak Identification Algorithm page:

.

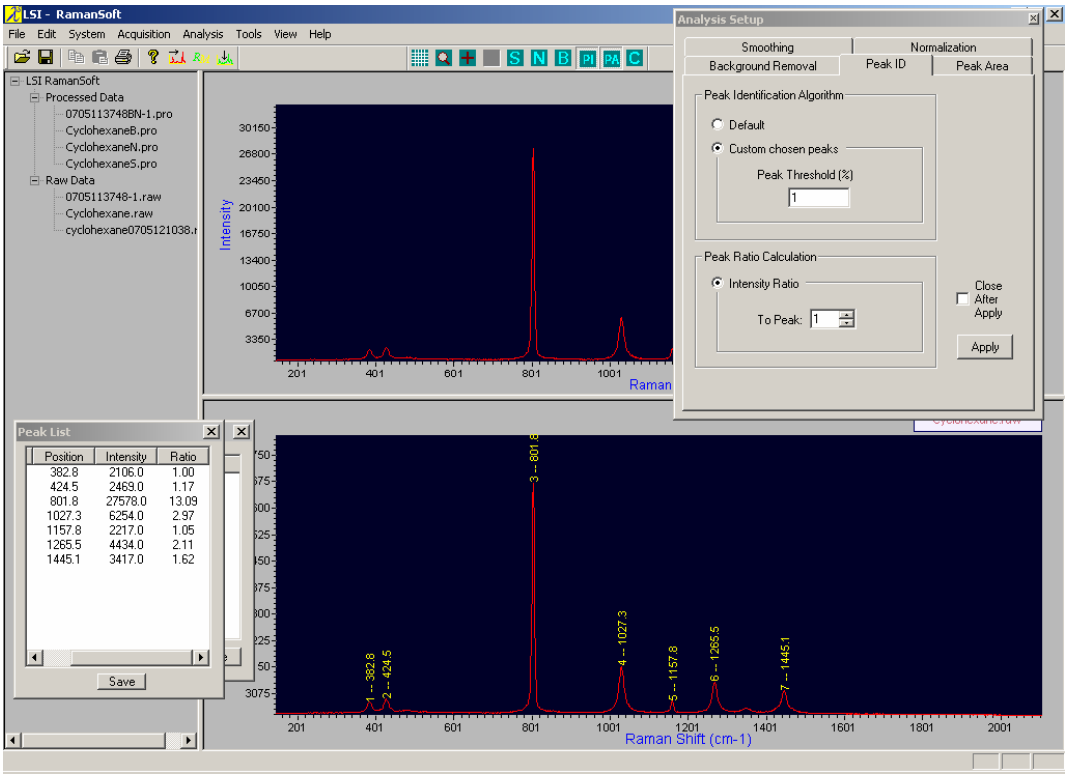


- Click on  in the Analysis Window Toolbar to clear the Analysis Window. In the File View Window, double click on the file name “Cyclohexane.raw” in the Raw Data list to bring the spectrum to the Analysis Window.

- Click *Apply* to apply *Custom chosen peaks* algorithm with 1% of peak threshold to the spectrum. This means that all peaks 1% or greater than the largest peak will be identified. The peak labeled spectrum is now displayed in the Analysis Window. Note that a peak table is also displayed, listing the peak position and peak intensity values along with an index number for each peak.

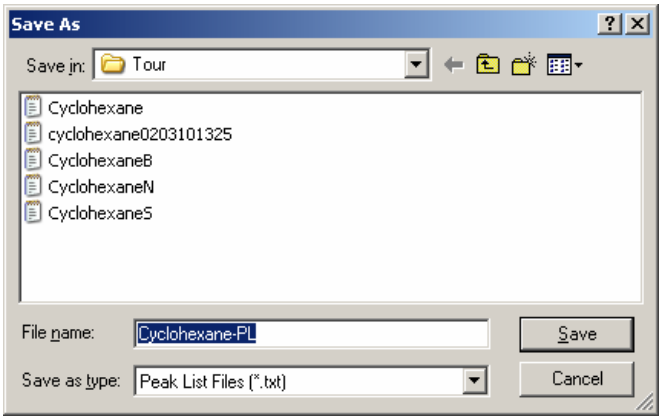


- To obtain the peak ratio of the identified peaks, click on the *Intensity Ratio* radio button under the *Peak Ratio Calculation* group box. The default algorithm is the ratio of the intensity of each peak to that of the first peak. Click Apply.



Note that in the *Peak List* table, a *Ratio* column lists the intensity ratio values.

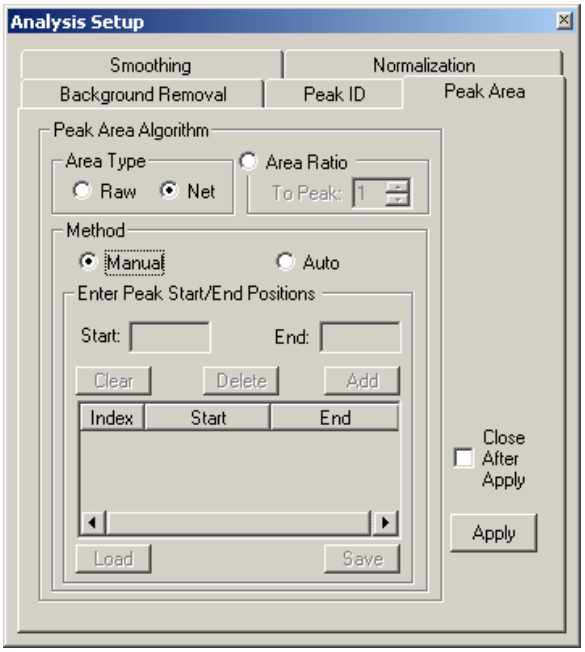
- The user can save the peak list by clicking Save on the Peak List Window. Click *Save* to save it into a text file. A default name (e.g., Cyclohexane-PL.txt) is provided by the program.



Peak Area / Area Ratio

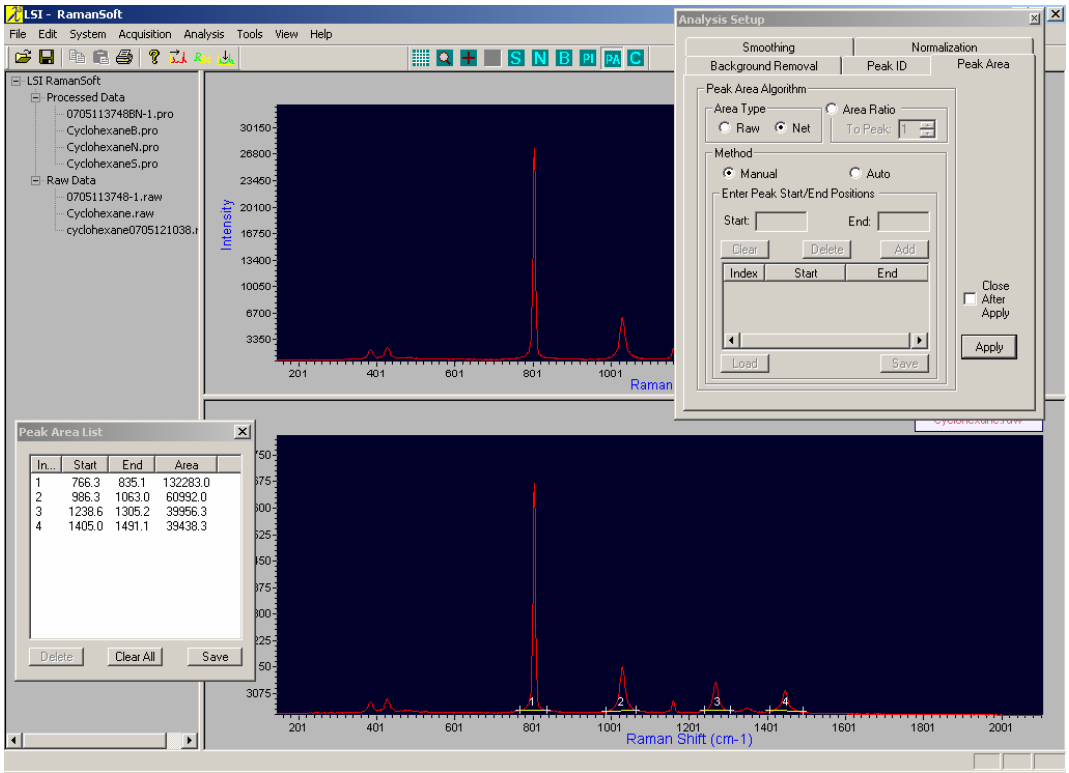
- Click on the *Peak Area* tab to bring up the Peak Area Algorithm page:

.

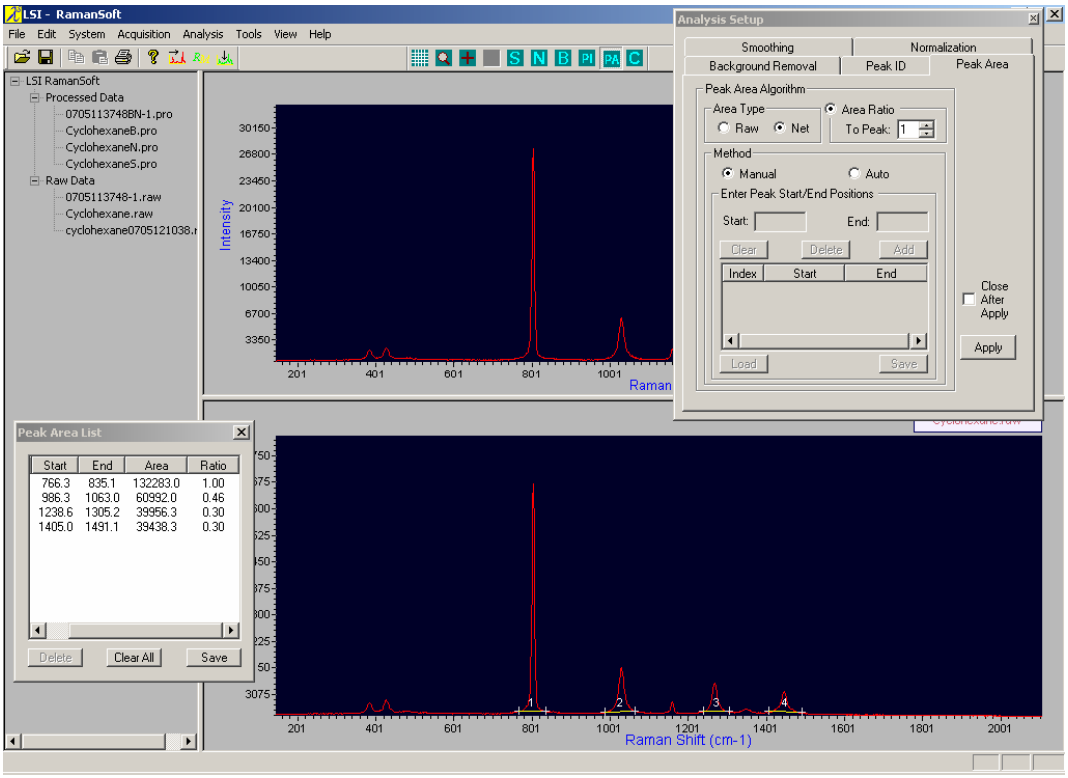


- Click on  in the Analysis Window Toolbar to clear the Analysis Window. In the File View Window, double click on the file name “Cyclohexane.raw” in the Raw Data list to bring the spectrum to the Analysis Window.

- *Manual* is the default method for peak area calculation. Click *Apply*, use cursor to draw a line at the base of the peak that the user regards as the baseline of the peak. Do the same to some other peaks. Right mouse click then gives a list of all the peaks labeled in the Analysis Window and all the areas are tabulated in the Peak Area List window.



- To obtain the peak ratio, click the *Area Ratio* radio button. The default algorithm is the ratio of the area of each peak to that of the first peak. Click Apply.



Note that in the *Peak Area List* table, a *Ratio* column lists the area ratio values.

- To save the peak area data listed in the Peak Area List window, click *Save* to save it into a text file. A default name (e.g., Cyclohexane-PA.txt) is provided by the program.

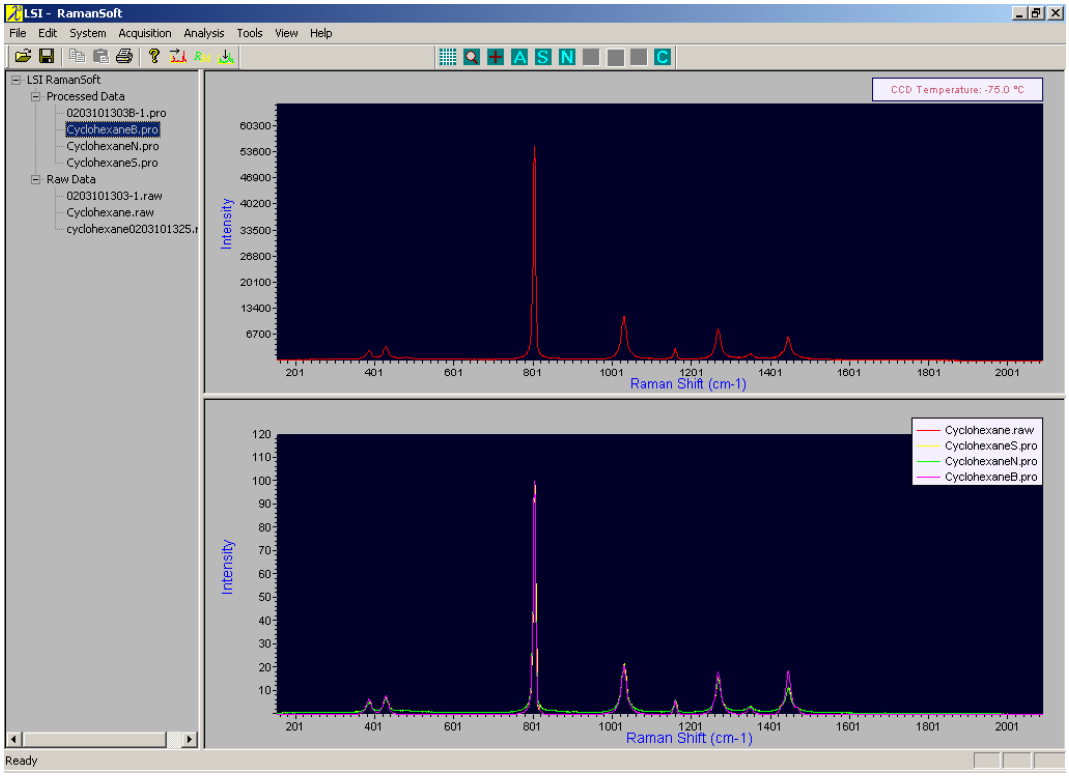
Spectrum Overlay

Spectrum Overlay allows multiple spectra to be plotted and viewed in the Analysis Window simultaneously.

- From *Analysis* menu, check *Spectrum Overlay*.

- Click on C in the Analysis Window Toolbar to clear the Analysis Window. From the Raw Data list in the File View Window, click on files “Cyclohexane.raw”, “CyclohexaneS.raw”, “CyclohexaneN.raw”, “CyclohexaneB.raw”. The spectra are overlaid using different colors.

- The overlaid spectra can be processed simultaneously using the smoothing or the normalization methods. Click N on the Analysis Window Toolbar to apply normalization to the spectra. All the spectra should be normalized to the maximum peak (the normalization algorithm used can be checked and changed by clicking A on the Analysis Window Toolbar and go to the Normalization page).



- Note that on the Analysis Window Toolbar, only S and N are available. Click C to clear the Analysis Window, and click *Analysis->Single Spectrum* to switch back to the single spectrum display mode.

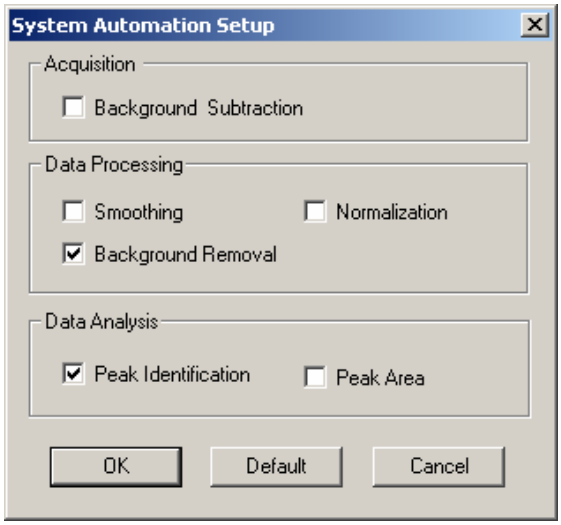
System Automation

All the data processing and analysis methods can be automated so that once a spectrum is acquired and displayed in the Acquisition Window, the pre-configured data processing/analysis method(s) can be applied to the acquired spectrum, and the processed spectrum is simultaneously displayed in the Analysis Window.

- From *Acquisition->Setup*, change the filename prefix to “cycloSA”.

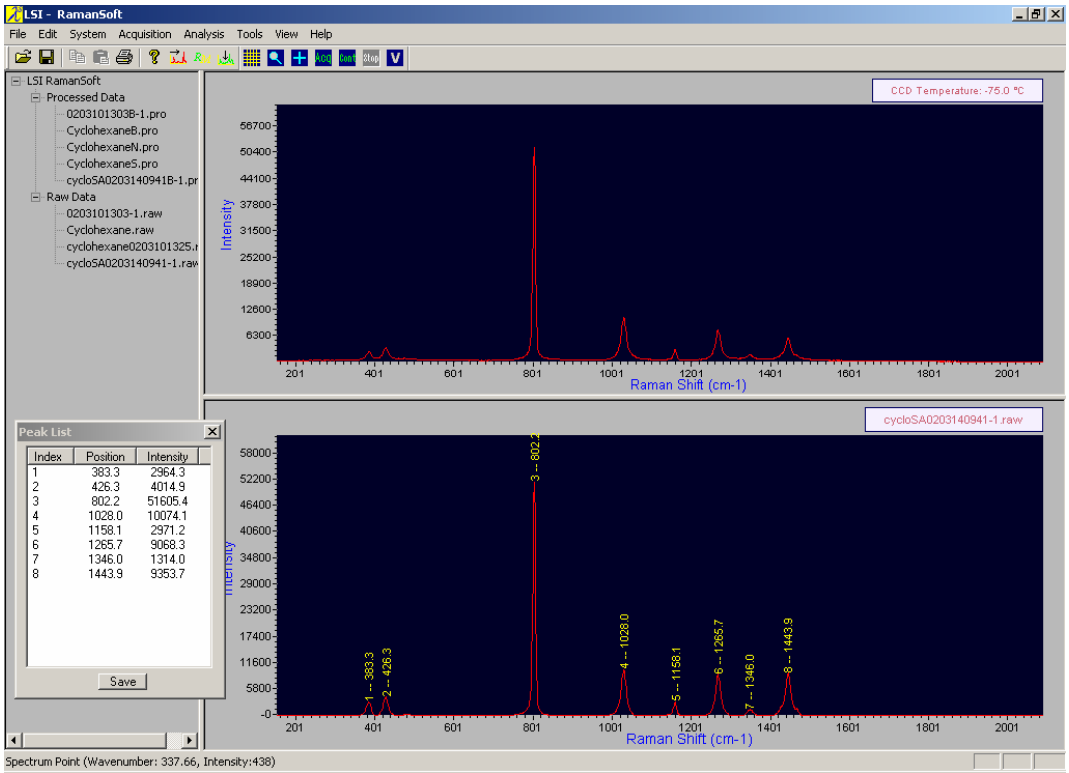
- From *Analysis->Algorithm Setup*, configure the algorithms desired. Set Background Removal and Peak ID algorithms as those in Section “Spectrum Processing / Analysis**”**.

- From *System->Automation->Setup*, check *Background Removal* and *Peak Identification*, and click OK.



- Click  on the Acquisition Window Toolbar to acquire a spectrum. If the Acquisition Window Toolbar is absent, click anywhere in the Acquisition Window to bring it up.

- The raw spectrum is displayed in the upper Acquisition Window, and the background-removed and peak-labeled spectrum is displayed in the lower Analysis Window.

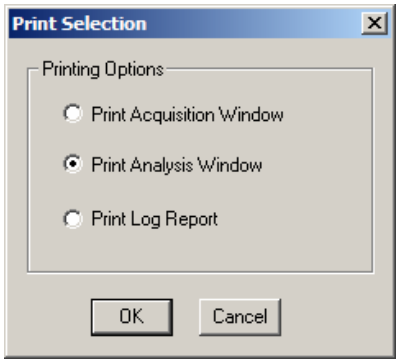


- Note that the processed spectrum is automatically saved with the filename prefix “cycloSA”, followed by a date and time string, and then by letter “B”, where “B” stands for background removal.

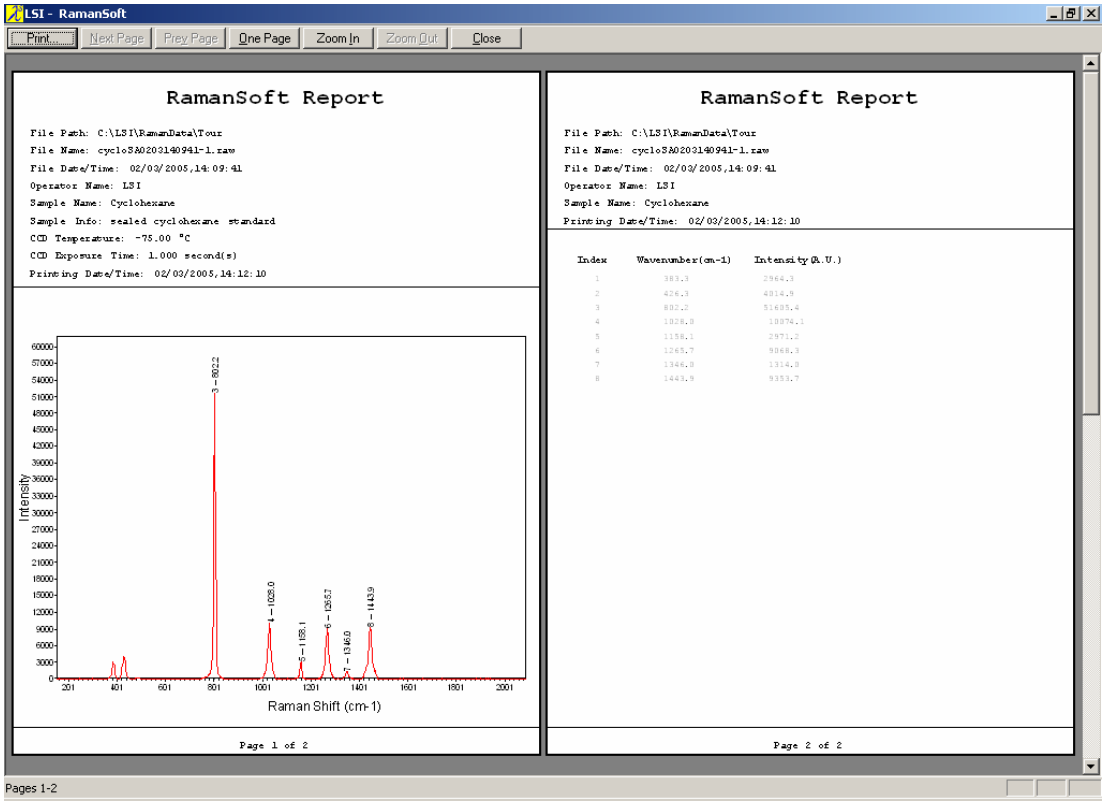
RamanSoft Report

The user can generate a hard-copy report of a data acquisition by printing out the spectrum and the system parameters associated with the data acquisition.

- Click on *File->Print Preview*, the Print Selection window appears:



- Check *Print Analysis Window* radio button, and click OK. The report is then shown in the print preview page.



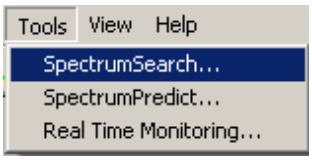
- Press “Print” to print the report to your designated printer.

Spectrum Searching

To identify an unknown spectrum through the integrated RamanSoft and GRAMS Spectral ID® use the SpectrumSearch interface. The spectrum can be searched against a spectral library containing spectra of known materials.

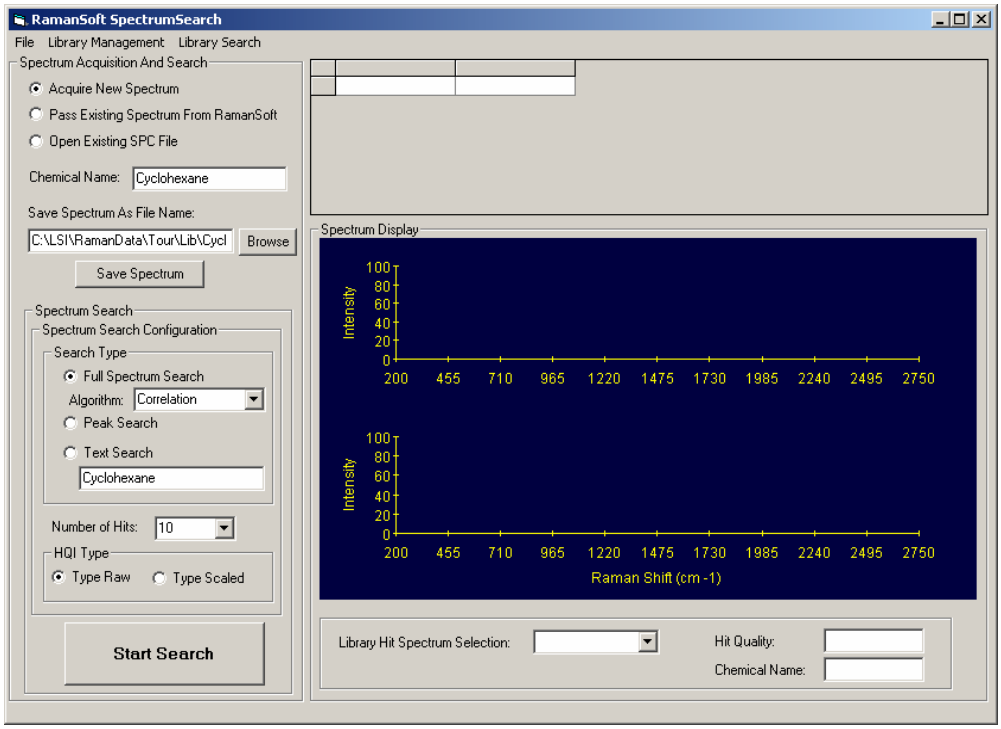
Launching the SpectrumSearch Application

- From the main menu, click *Tools->SpectrumSearch*.



Alternatively, the user can click on the SpectrumSearch icon on the Function Toolbar:

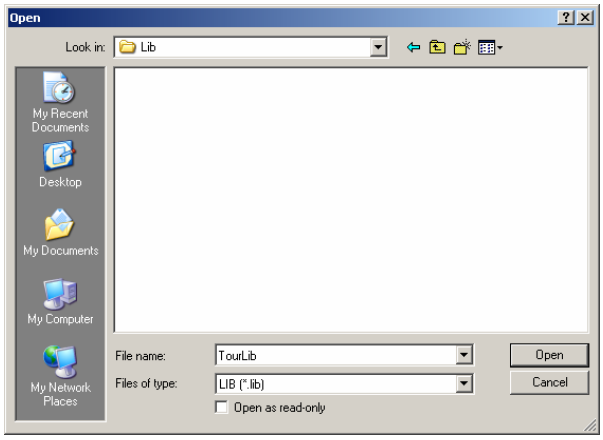
- The SpectrumSearch user interface appears.



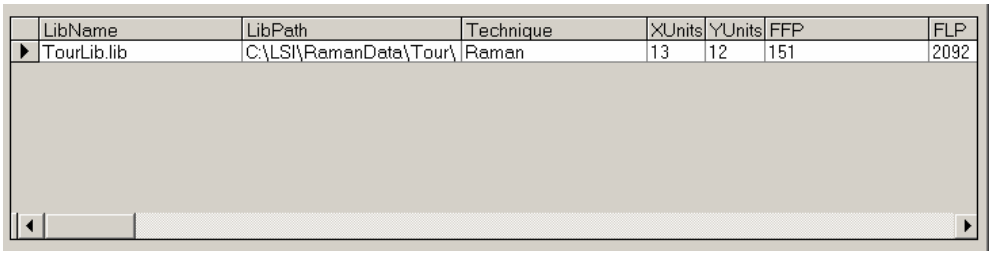
Note: For users who want to skip the library creation procedure and instead go directly to search a library, please proceed to section “Select and View Library”.

Creating a New Library

- Create a subfolder \Lib within the \Tour folder: C:\LSI\RamanData\Tour\Lib.
- From SpectrumSearch main menu, click *Library Management->Create New Library*.
- Browse to C:\LSI\RamanData\Tour\Lib and enter the name of the library “TourLib”.



- Now the library is created but empty. The name of the library is displayed in the top right section of the SpectrumSearch user interface.



Acquiring a Library Spectrum

The spectra for library creation can be acquired either from RamanSoft main interface (see Section “Acquire a Spectrum” in this Tour) or from the SpectrumSearch interface. This section describes the method to acquire a library spectrum from the SpectrumSearch interface.

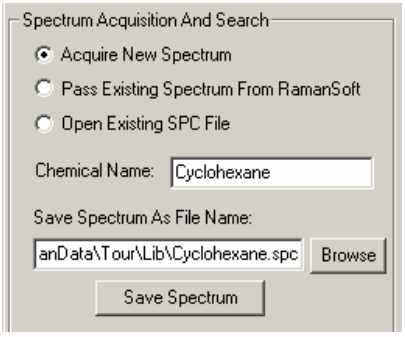
- From RamanSoft main menu, choose *System->CCD Configuration*. Set the desired integration time.
- From RamanSoft main menu, choose *Acquisition->Setup*. Set the desired repeats for each acquisition in the *Frames Per Measurement* textbox. In the *Path* field under *File Auto Save*, enter C:\LSI\RamanData\Tour\Lib\LibData. Enter sample name and file prefix. Note that the files in

\LibData are not used for library creation, but as they are in LSI format, they can serve as a future reference for the library spectra.

- From RamanSoft main menu, choose *Analysis->Algorithm Setup*. Go to the Background Removal page. Make sure that the *Automatic* radio button is checked.

- From RamanSoft main menu, choose *System->Automation->Setup*. Make sure that (only) the *Background Removal* checkbox is checked. This is to ensure that the spectra acquired and entered into the library will have a flat background.

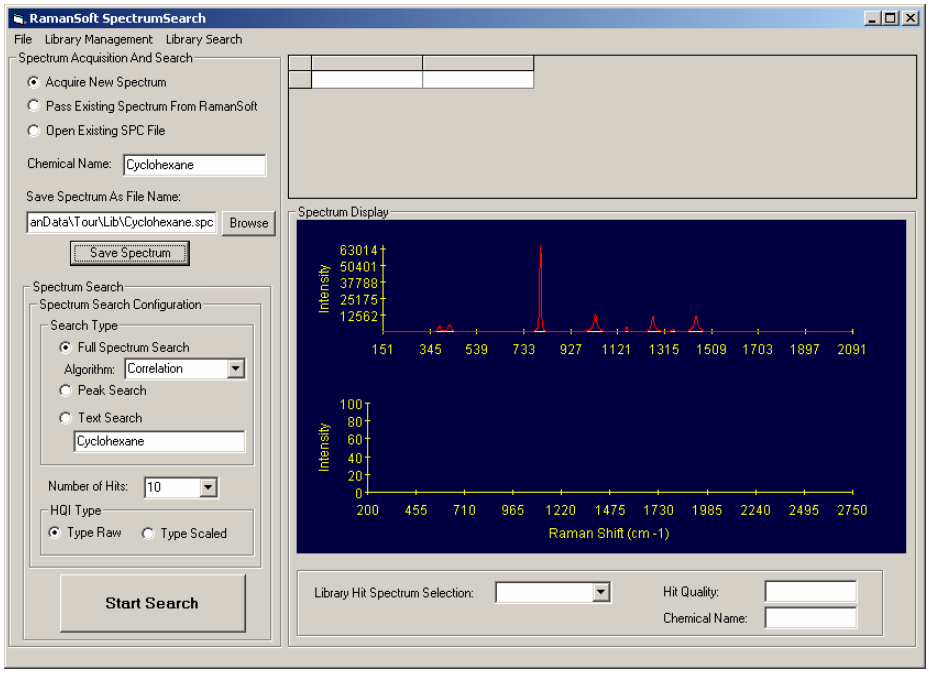
- In *Spectrum Acquisition and Search* window, choose *Acquire New Spectrum*.



- In the *Chemical Name* field, enter the name of the sample, for example, Cyclohexane. Be sure to enter a meaningful name for the sample, as this is the name of the sample that is to be stored in the library when the spectrum is added to the library and that is to be retrieved from the library when a search is performed.

- In *Save Spectrum As File Name* field, enter the file name and path where the library spectrum is going to be saved, for e.g., C:\LSI\RamanData\Tour\Lib\Cyclohexane.spc. The saved SPC files are to be used for library creation.

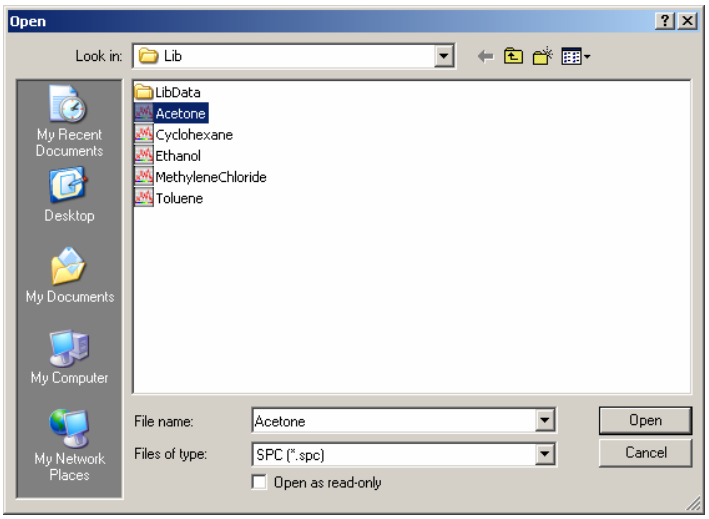
- In *Spectrum Acquisition and Search* group box, click *Save Spectrum*. The spectrum is acquired and saved.



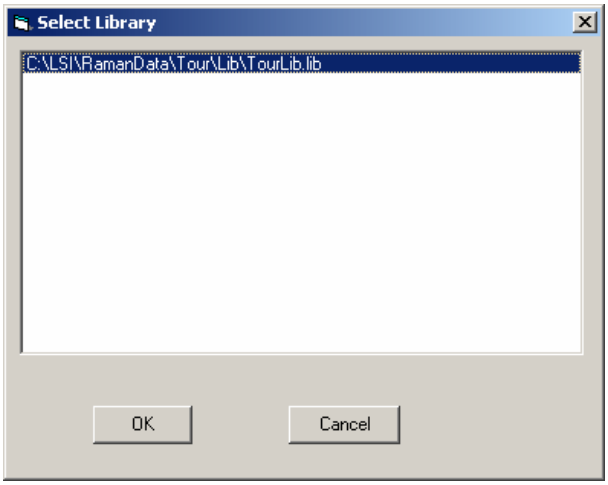
- Repeat this procedure to acquire other library spectra.

Adding a Spectrum to the Library

- Click on *Library Management->Add New Spectrum*. Browse to C:\LSI\RamanData\Tour\Lib where the library spectra were saved. Select the filename (in GRAMS SPC format), and click OK.



- The Select Library window appears. Highlight the library where the spectrum is going to be added.

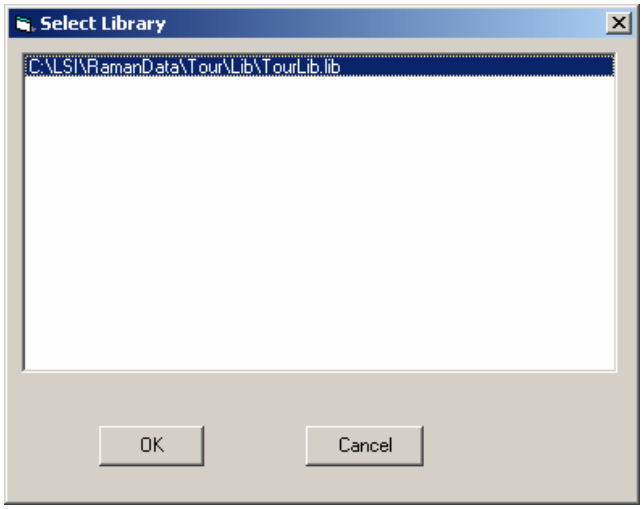


- The spectrum is now added to the library. On the top right section of the SpectrumSearch interface, only a *Row Status* is shown.

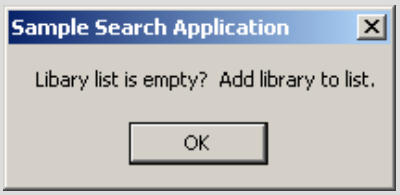
- Repeat this procedure to add the rest of the spectra into the library.

Selecting and Viewing Library

- From the main menu of SpectrumSearch module, choose *Library Management->View Library*.
- The Select Library window appears. Highlight the library item C:\LSI\RamanData\Tour\Lib \tourlib.lib. Click *OK*.

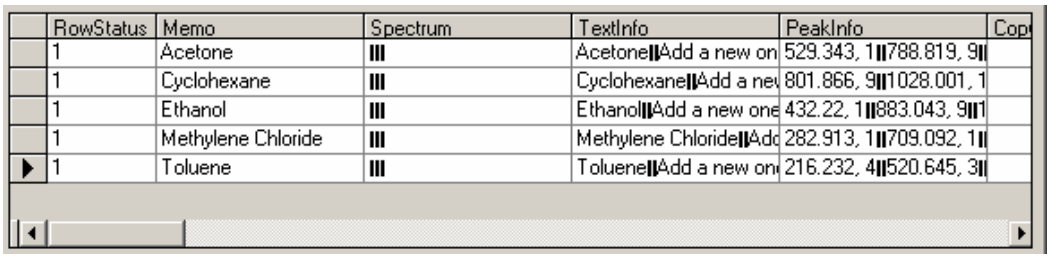


Note: For users who skipped Library Creation and had proceeded directly to here from subsection "Start SpectrumSearch Application", the following window may appear.



Click *OK*, go to *Library Management->Add Library to List*, browse to the directory C:\LSI\RamanData\Tour\Lib, select file "TourLib", and click *Open*.

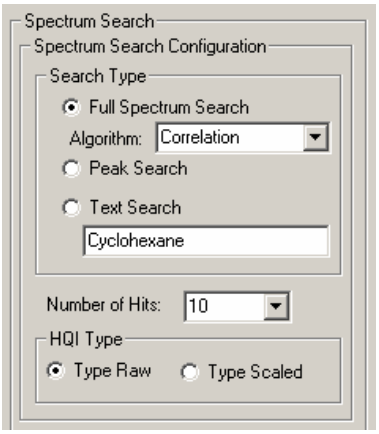
- All the library entries are displayed on the top right hand section of the SpectrumSearch interface.



	RowStatus	Memo	Spectrum	TextInfo	PeakInfo	Cop
	1	Acetone	III	Acetone‖Add a new on	529.343, 1‖788.819, 9‖	
	1	Cyclohexane	III	Cyclohexane‖Add a ne	801.866, 9‖1028.001, 1	
	1	Ethanol	III	Ethanol‖Add a new one	432.22, 1‖883.043, 9‖1	
	1	Methylene Chloride	III	Methylene Chloride‖Adc	282.913, 1‖709.092, 1‖	
▶	1	Toluene	III	Toluene‖Add a new on	216.232, 4‖520.645, 3‖	

Setting Search Configuration

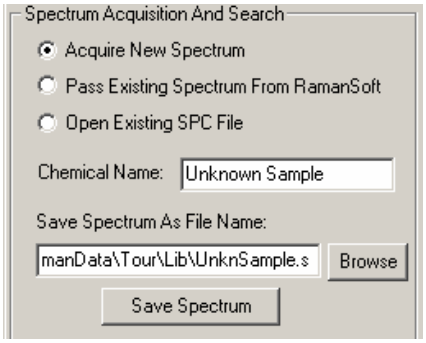
- In the *Spectrum Search* section of the SpectrumSearch main interface, select the desired algorithm (the default algorithm is *Correlation* algorithm) to perform a full spectrum Search, leave other options in their default settings.



Performing a Spectrum Search

- Put an “unknown” sample (e.g., cyclohexane as supplied or any other sample) into the internal sample cell or External Sampling Module. From RamanSoft main menu, set acquisition parameters such as CCD integration time (this can be left as 1 sec). From *System->Automation->Setup*, check *Background Removal*. Uncheck other options. In *Acquisition->Setup*, set the number of frames to repeat (this can be left as 1). Type in file *Path* as “C:\LSI\RamanData\Tour\Lib\LibData”, *Sample Name* as “Unknown Sample”, *File Prefix* as “UnknSample”.

- Under *Spectrum Acquisition and Search*, type in *Chemical Name* as “Unknown Sample”.

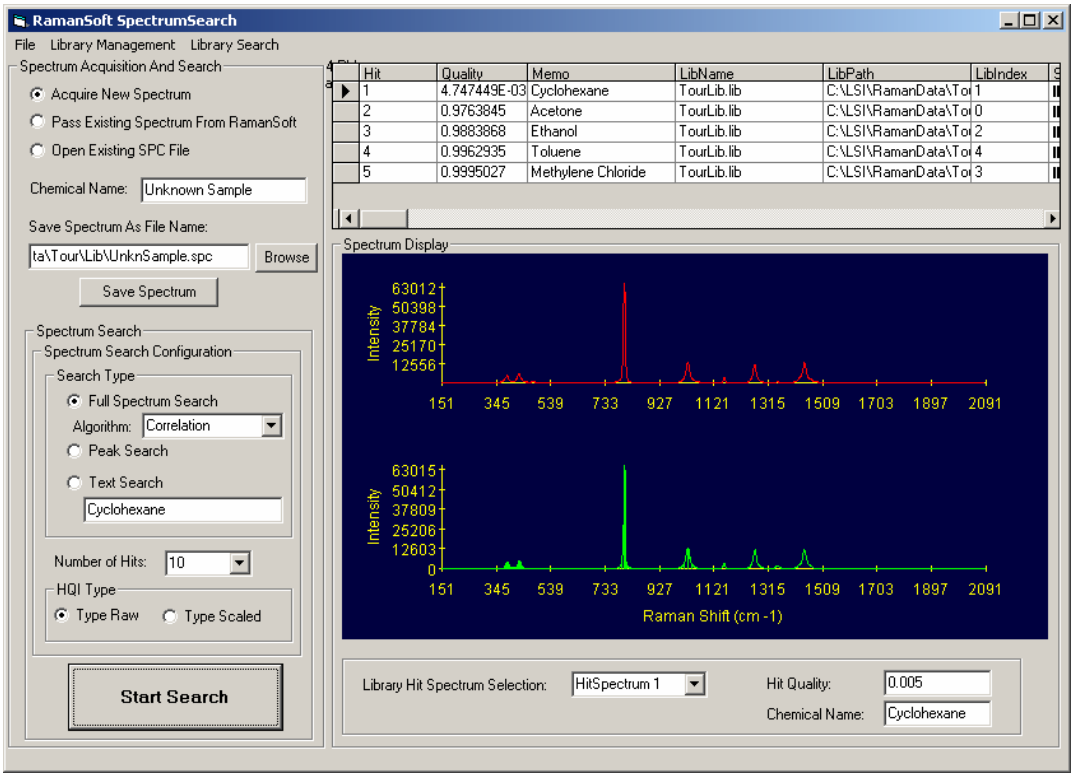


Note: The laser may turn off automatically if the system has been idle for more than 30 minutes. Make sure that the laser is on by checking *System->Laser Setting->Laser On* before pressing *Start Search*.

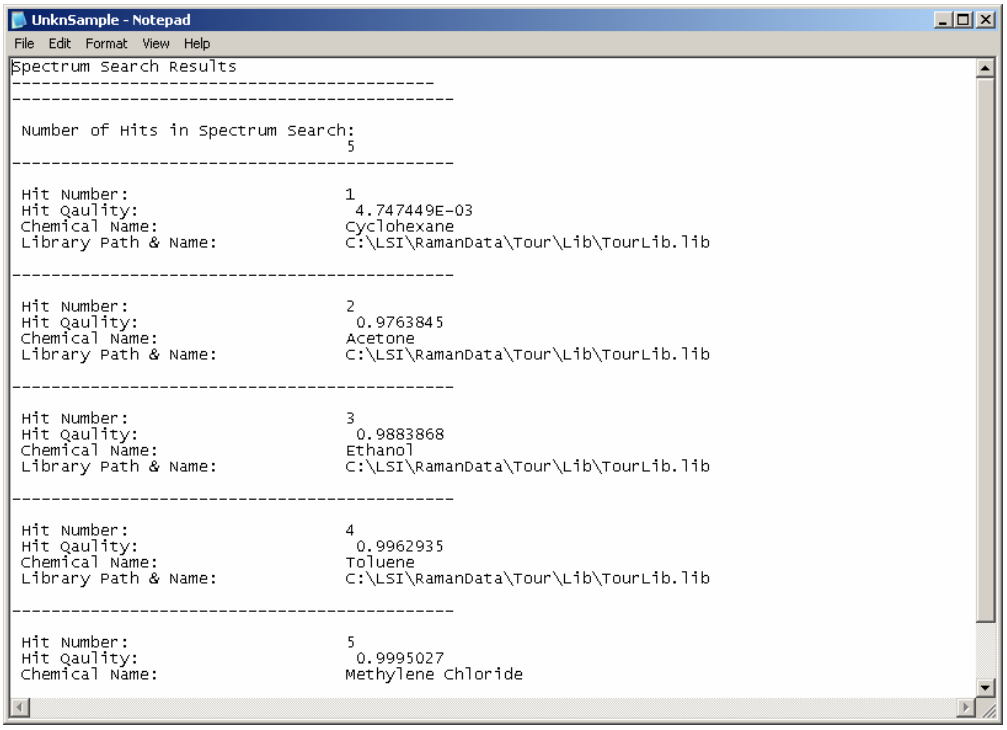
- Press *Start Search* to start spectrum acquisition followed by spectrum searching, in one-step.

Viewing Search Results

- On the SpectrumSearch interface, the top right section lists the matched library items in a decreasing “goodness-of-match” order. The middle right section displays the spectrum of the unknown sample (top plot) and the spectrum of the best match library spectrum (lower plot). In the lower right section, other matched spectra can be viewed through the *Library Hit Spectrum Selection* pull-down selection, along with the score (*Hit Quality*), as well as the *Chemical Name* of the matched library spectrum. The figure shows the search results of the “unknown” sample cyclohexane. Note that in the *Type Raw* scoring scheme, the lower the score, the better the match. For the tour example shown below, the match score was 4.747449E-03, or ~ 0.005, which indicates an excellent match.



- The search results are also saved in a text file located in the same folder as the library spectrum. For e.g., C:\LSI\RamanData\Tour\Lib\UnknSample.txt.



- From SpectrumSearch interface, Click *File->Exit* to exit the SpectrumSearch application.

Predicting a Spectrum

To predict an unknown spectrum either qualitatively (find a match to a known group of samples) or quantitatively (find the composition of a sample of known ingredients) using the integrated RamanSoft and GRAMS IQ Predict™ interface SpectrumPredict, a calibration file needs to be pre-established through GRAMS/AI™ with PLSplus/IQ™ add-on. An unknown sample spectrum can then be acquired and predicted through SpectrumPredict.

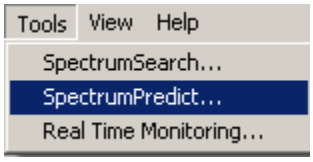
Building a Calibration File

- For the tour purpose, a calibration file (Cal file) has been pre-built (this is also necessary for customers who opt to get Thermo Electron's IQ Predict™ alone instead of the full package of GRAMS/AI™ with PLSplus/IQ™ add-on). The Cal file, along with the training data set that was used to build the Cal file, is located at C:\LSI\RamanData\Tour\Predict. The training data set consists of 11 samples of cyclohexane (Constituent 1) and methylene chloride (Constituent 2) at various proportions. Note that for real applications, much larger sample sizes (25 samples or above) are recommended to create more refined calibration files and thus to obtain more precise predictions.

- For users who would like to build their own calibration file, please refer to Thermo Electron's PLSplus/IQ™ User Guide to do so.

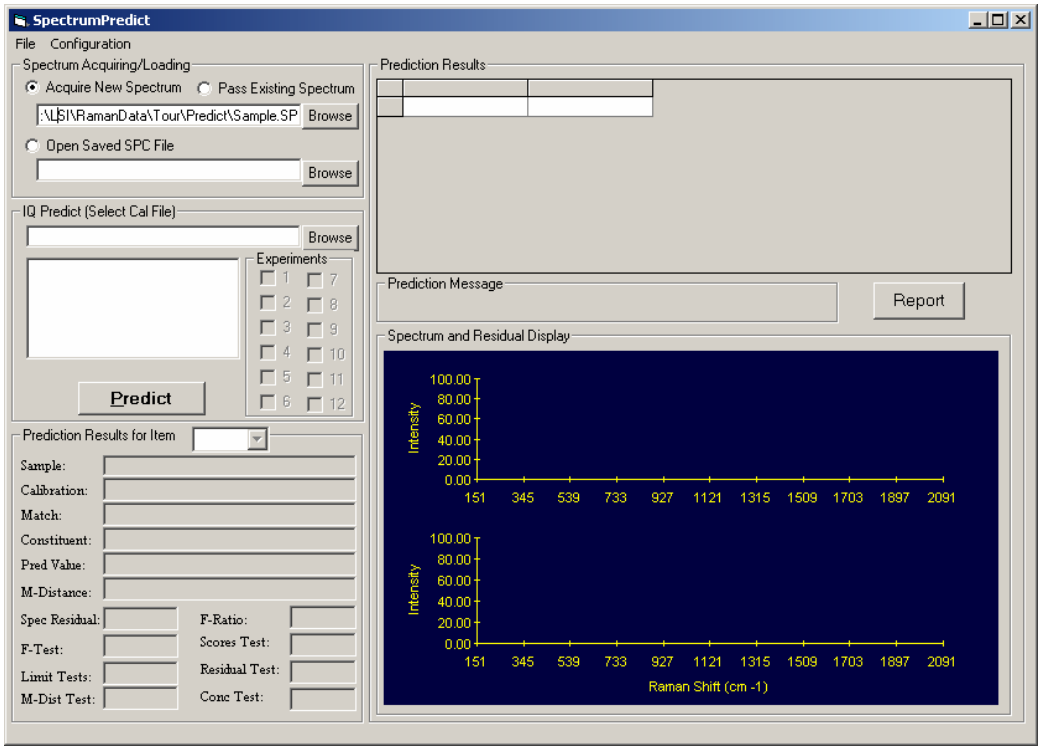
Launching the SpectrumPredict Application

- From RamanSoft main menu, click *Tools->SpectrumPredict*.



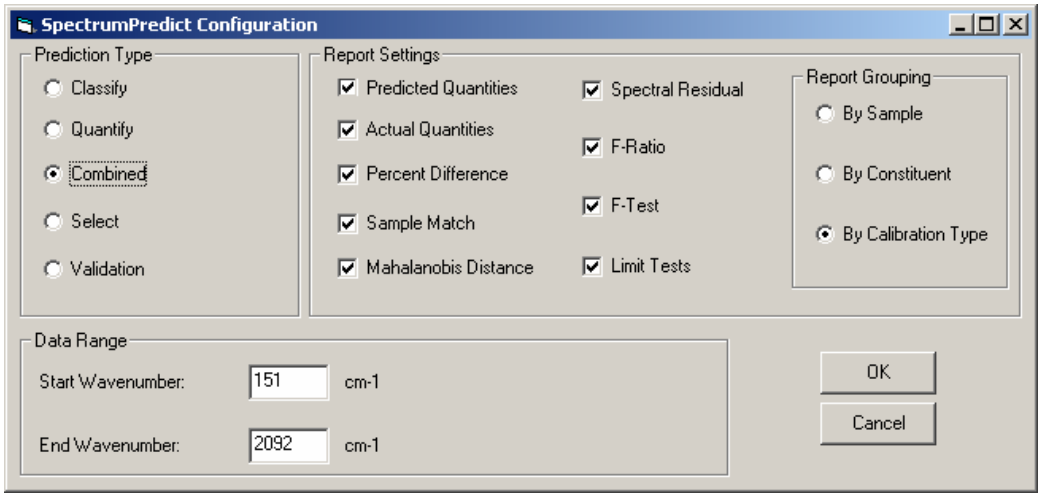
Alternatively, the user can click on the SpectrumPredict icon on the Function Toolbar:

- The SpectrumPredict user interface appears:



Configuring Prediction Settings

- From the SpectrumPredict menu, choose *Configuration->SpectrumPredict Settings*. The SpectrumPredict Configuration window appears. Accept the default settings by clicking *OK*.



Configuring Spectrum Acquisition Parameters

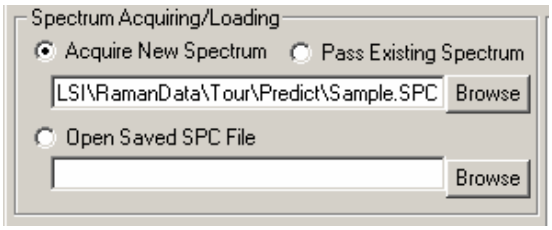
- From RamanSoft main menu, choose *System->CCD Configuration*. Set the desired integration time. For the tour, the integration time can be left as 1 sec.

- From RamanSoft main menu, choose *Acquisition->Setup*. Set the desired number of frames to repeat (can be left as 1) for each acquisition in the *Frames Per Measurement* textbox. In the *Path* field under *File Auto Save*, enter C:\LSI\RamanData\Tour\Predict. Type in *Sample Name* and *File Prefix* as “Sample”.

- From RamanSoft main menu, choose *Analysis->Algorithm Setup*. Click on the Background Removal page tab. Make sure that the *Automatic* radio button is checked, and click the *Apply* button.

- From RamanSoft main menu, choose *System->Automation->Setup*. Make sure that the *Background Removal* checkbox is checked (and that no other options are checked). This is to be consistent with the fact that the Cal file is built with the automatic background removal feature turned on.

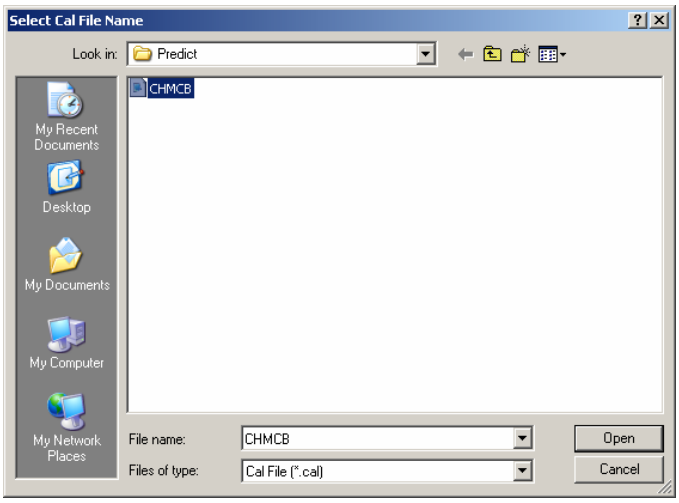
- From SpectrumPredict interface, check *Acquire New Spectrum* under *Spectrum Acquiring / Loading*. Type in the file path/name as C:\LSI\RamanData\Tour\Predict\Sample.spc.



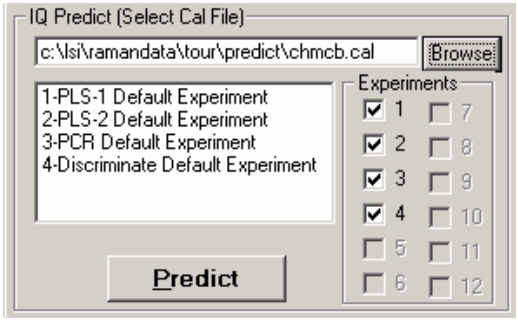
Loading Calibration File and Performing Spectrum Prediction

- From SpectrumPredict interface, click *Browse* button under *IQ Predict* (*Select Cal File*).

- The Select Cal File Name window appears. Browse to C:\LSI\RamanData\Tour\Predict to locate the calibration file (CHMCB.cal). Click *Open*.



- The calibration methods (experiments) built in the calibration file are loaded and displayed in the text window.

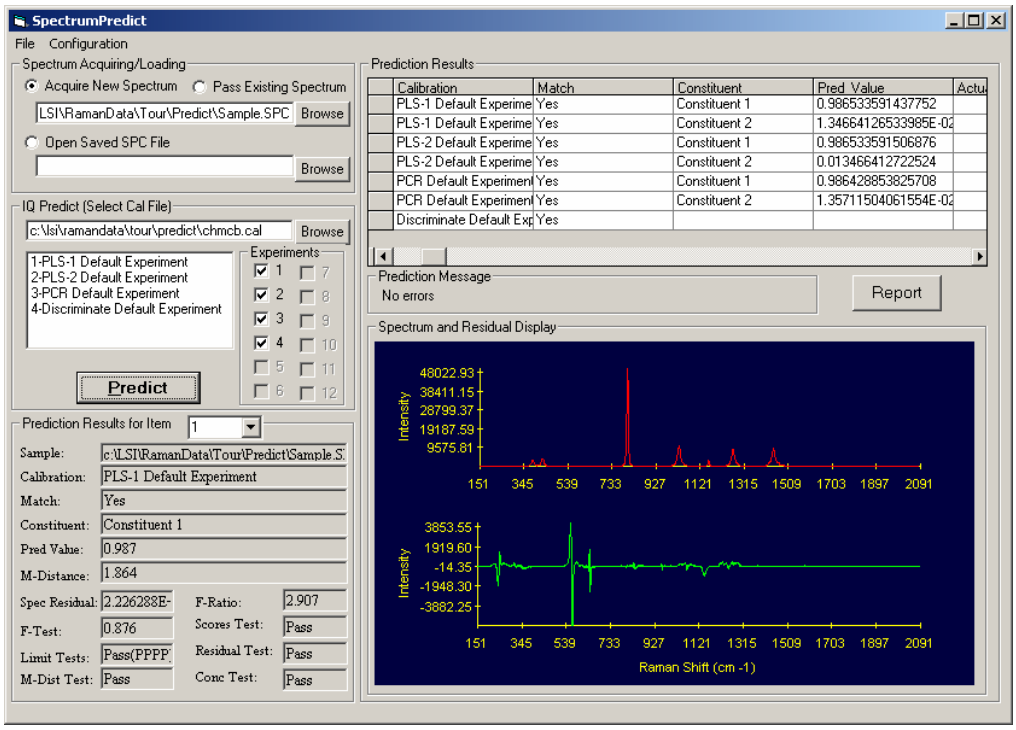


Note: The laser may turn off automatically if the system has been idle for more than 30 minutes. Make sure that the laser is on by checking *System->Laser Setting->Laser On* before pressing *Predict*.

- Put the “unknown composition” sample cyclohexane (or a mixture of cyclohexane and methylene chloride if they both are available; please note these are volatile solvents and thus care should be taken in order to prepare defined compositions) in the internal sample cell or External Sampling Module. Press *Predict* to perform spectrum prediction.

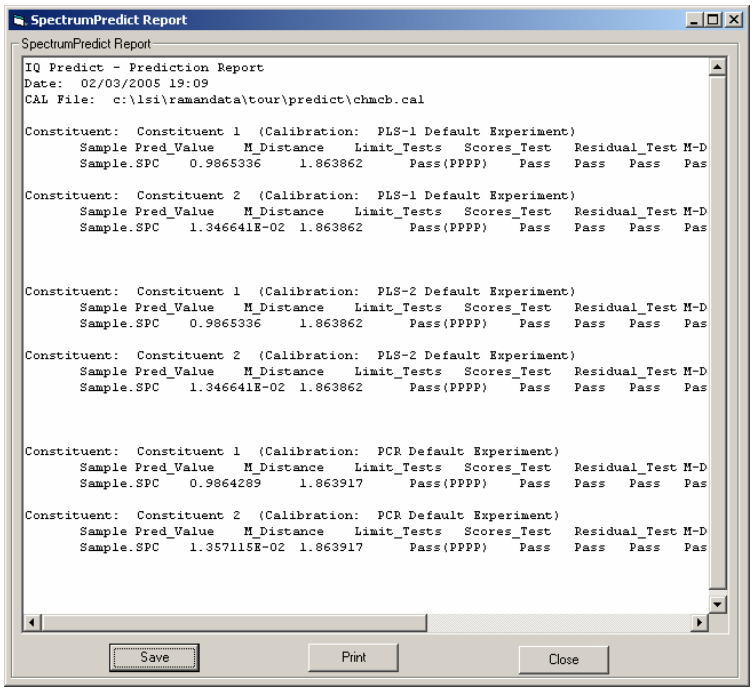
Viewing Prediction Results

- The prediction results are displayed in the SpectrumPredict main interface.



The top right section shows the itemized prediction results for each constituent in the sample by each calibration method. The bottom right section displays the sample spectrum (top panel) and the residual spectrum (bottom panel). The bottom left section displays all the prediction results for each item, where an item corresponds to a row in the top right section of the interface.

- The prediction results can also be viewed by pressing *Report* on the SpectrumPredict interface. The results can be saved as Rich Text File (RTF) or as plain text file (.txt), or can be printed out.



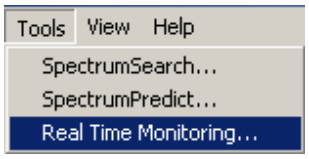
- From SpectrumPredict interface, click *File->Exit* to exit the SpectrumPredict application.

Monitoring a Process in Real Time

To monitor a process in real time, a peak (or up to five peaks) of interest need to be identified first from the spectrum of the sample of interest. Either the peak intensity or the peak area can then be followed as a function of time.

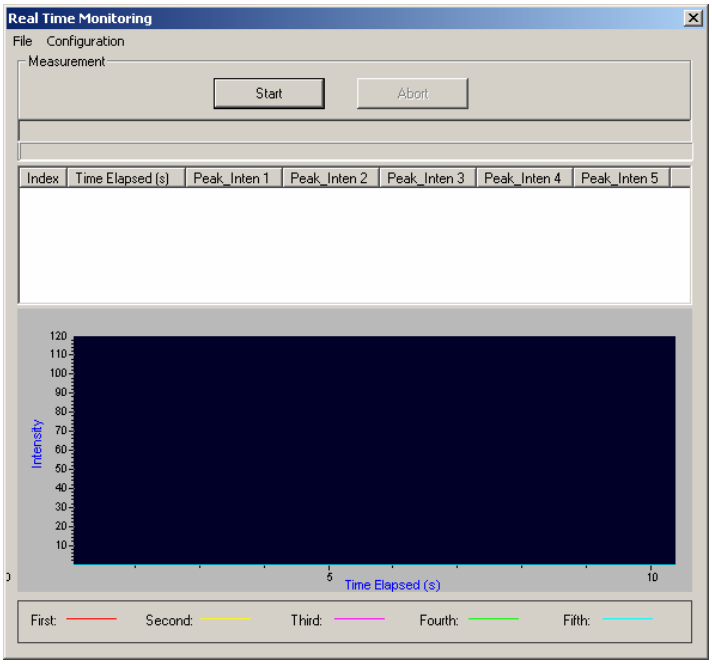
Launching Real Time Monitoring Application

- From the main menu, choose *Tools->Real Time Monitoring…*.



Alternatively, the user can click on the Real Time Monitoring icon on the Function Toolbar:

- The Real Time Monitoring main interface appears as follows.

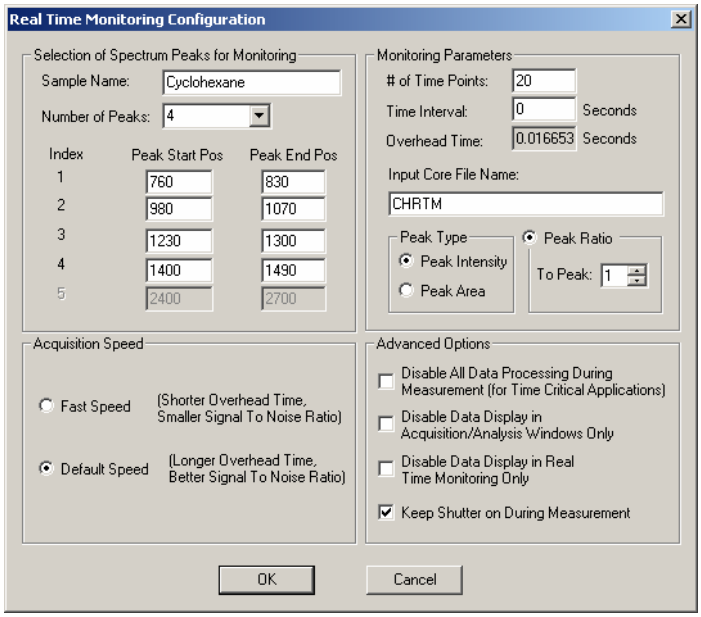


Setting Acquisition Parameters

- From RamanSoft main menu, choose *System->CCD Configuration*. Set the desired CCD integration time. For the tour, the integration time can be left as 1 sec.
- From RamanSoft main menu, choose *System->Automation->Setup*. Uncheck the *Background Removal* option or other options.
- From RamanSoft main menu, click *System->Automation->On*. This is to enable auto file saving when no option is selected in the Automation Setup window.
- From RamanSoft main menu, choose *Acquisition->Setup*. Set the desired frames to average for each acquisition in the *Frames Per Measurement* textbox as 1. In the *Path* field, enter C:\LSI\RamanData\Tour\RTM. This is the directory where the real time monitoring data will be saved. Enter *Sample Name* and *File Prefix* as “Cyclohexane”.
- Put the sealed cyclohexane standard sample in the sample cell. Click *Acquisition->Acquire* or  on the Acquisition Window Toolbar to acquire a cyclohexane spectrum.
- Find the start and end positions of the peaks to be monitored.

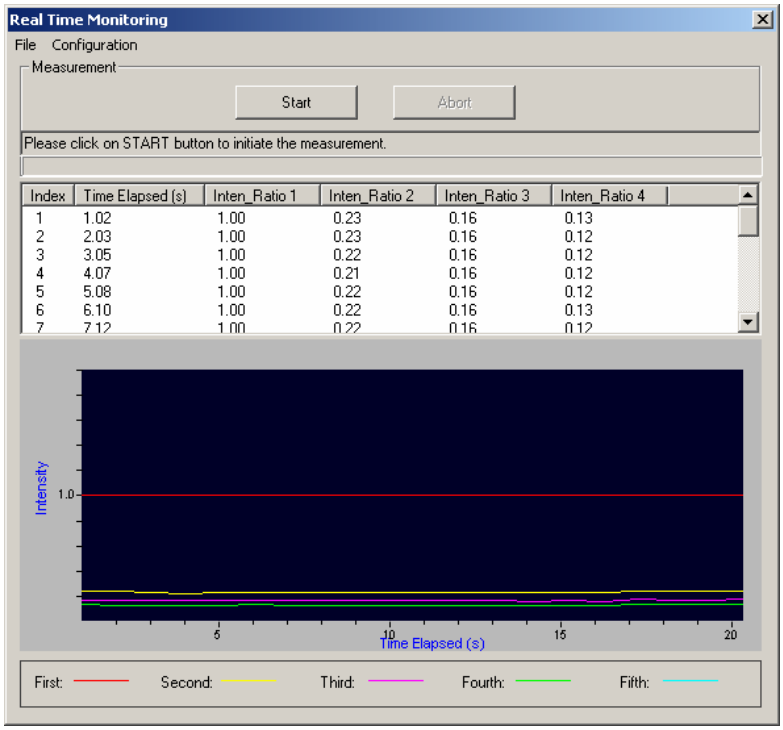
Setting Real Time Monitoring Configuration

- From the Real Time Monitoring main menu, choose *Configuration->Configuration Setup*.
- Enter the *Sample Name*, select the *Number of Peaks* to follow, and enter the *Peak Start Pos* and *Peak End Pos* for each peak. Use *Default Speed* as the acquisition speed.
- Enter *# of Time Points* as 20, *Time Interval* as 0 seconds. Type in the core file name in the *Input Core File Name* textbox. This core file name is to be followed by an index number at each time point, which together makes up the filename for the spectrum at each time point. Check the *Peak Intensity* and *Peak Ratio* radio buttons. As the time interval between consecutive monitoring points is zero, it is recommended that the *Keep Shutter on During Measurement* is checked.



Starting Real Time Monitoring

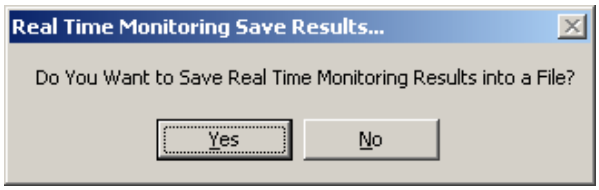
- From the Real Time Monitoring interface, press *Start*.
- The progress of the monitoring process can be viewed through the progress bar. The peak intensity ratio values for each of the monitored peaks are tabulated in the text window with the *Index* indicating the sequential time points and *Time Elapsed* displaying the actual time elapsed since the monitoring was initiated. The peak intensity ratio values for each of the monitored peaks are plotted in the bottom plot window at each time point



- Note that at the end of the real time monitoring, RamanSoft takes one additional acquisition of the sample using the settings prior to Real Time Monitoring application to restore the parameter settings for RamanSoft.

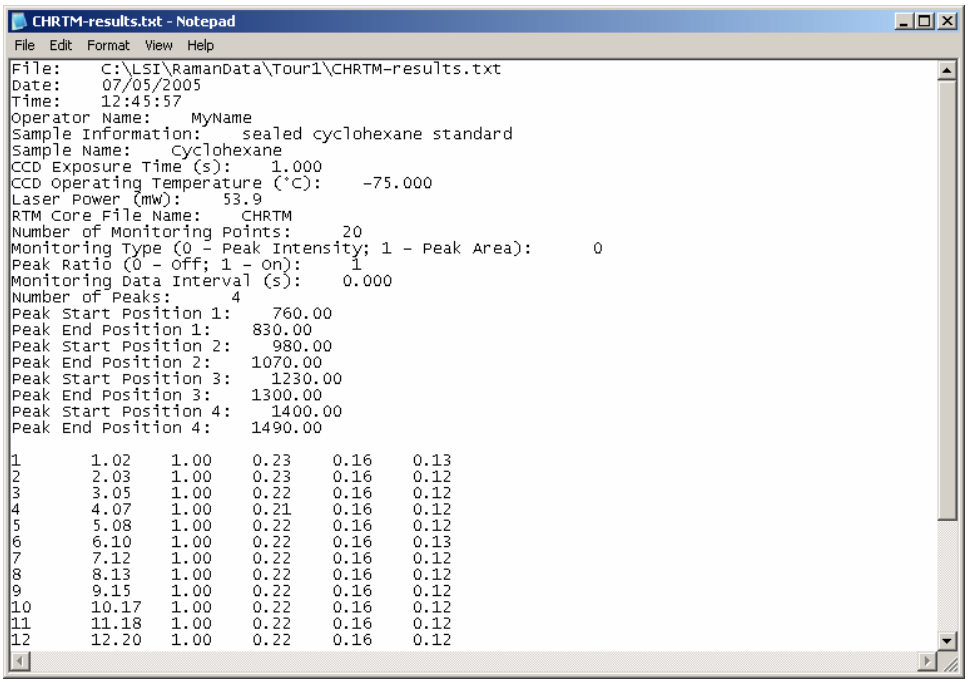
Saving Monitoring Results

- When the real time monitoring session is finished, the user is prompted to save the monitoring results (the peak intensity/area values vs. time elapsed) into a text file.



This text file can later be loaded into the Real Time Monitoring interface to view the results.

- Click on *Yes* to save the results in the same folder as the Real Time Monitoring data file, for e.g., C:\LSI\RamanData\Tour\RTM\CHRTM.txt.

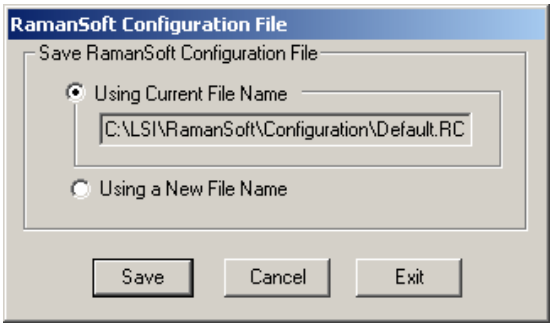


- From the Real Time Monitoring menu, choose *File->Exit* to exit the Real Time Monitoring application.

Exiting RamanSoft

- Make sure that all three function modules (SpectrumSearch, SpectrumPredict, and Real Time Monitoring) are exited.

- From RamanSoft main menu, choose *File->Exit*. The user is asked whether to save the configuration used in the RamanSoft session *Using Current File Name* (i.e., Default.rcf) or *Using a New File Name*.



- Check *Using a New File Name* radio button to save the configurations as your personal configuration file to be used for a future RamanSoft session.

- Press *Save*. The Save As window appears. Browse to C:\LSI\RamanSoft\Configuration (where the Default.rcf configuration file is located), and input the new configuration file name.
- Click *Save* in the Save As window. The new configuration file is then created and RamanSoft is exited.

Turning off Dimension-P Raman

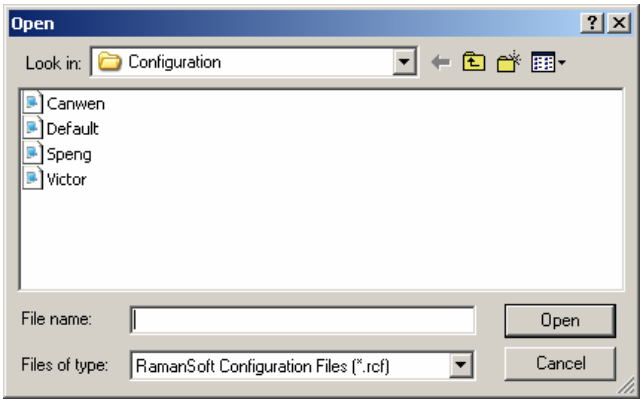
- Turn off the computer.
- Turn the Laser Power key to the **OFF** position.
- Turn off the power switch of Dimension-P Raman on the back panel of the unit.

5 RamanSoft Basic Functionalities

Upon RamanSoft Start-up

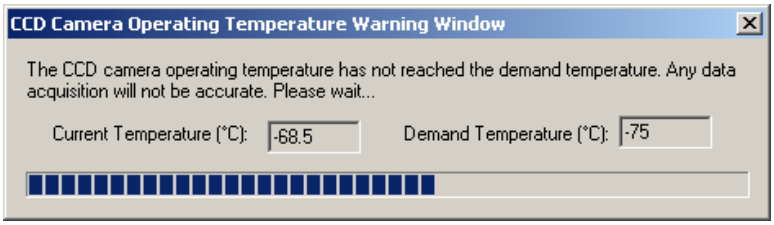
Selecting a Configuration File

When RamanSoft is first started, it asks the user to select a RamanSoft configuration file (.rcf file). This file contains settings that the user had previously used in RamanSoft, such as the configurations for data acquisition, data processing, and data analysis. If the user hasn't had a configuration file yet, the *Default.rcf* file can be used. *Default.rcf* contains default settings pre-configured by the program. Upon exiting RamanSoft, the user can save the configurations used in the RamanSoft session into a new file.



CCD Operating Temperature Warning Window

When RamanSoft is first started or when a new demand temperature is set, a temperature progress status bar will appear to inform the user when the desired temperature is reached.

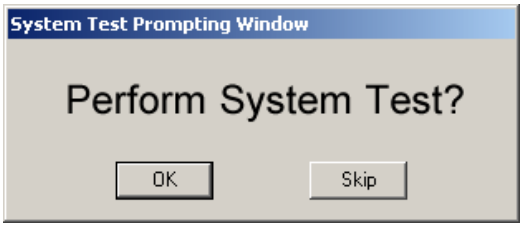


Laser Power Stabilizing Window

When the system is first started or when the laser is turned on, a Laser Power Stabilizing Progress Window is brought up to warn the user to wait (for about 15 seconds) for the laser to stabilize. Note that RamanSoft automatically turns the laser off when the system has been idle for more than 30 minutes. The system automatically turns the laser on when an acquisition starts.

System Test Prompting Window

When the system is first started, the System Test Prompting Window appears. A system test checks whether the instrument needs to be recalibrated or not by comparing the acquired spectrum from the sealed cyclohexane standard sample to the standard cyclohexane spectrum stored in the system. The user can opt to perform or skip the system test.

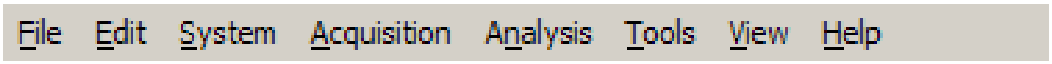


If the user clicks *OK* , a system test dialog window will then appear to allow the user to perform a system test, and the user will get test results instantly to decide whether the system needs to be recalibrated or not (for details, see Section “System Test” in this chapter). If the user clicks *Skip*, the user can then proceed to use the system assuming that the system stays calibrated and do not need a re-calibration.

The Main Menu, System Toolbars, and Frame Status Bar

The Main Menu

The main menu contains *File*, *Edit*, *System*, *Acquisition*, *Analysis*, *Tools*, View, and *Help* menu items. The function of each is described in detail in the following sections.

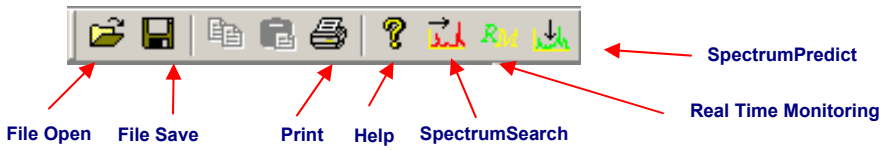


System Toolbars

The full system toolbar includes the Function Toolbar, the Acquisition Window Toolbar, and the Analysis Window Toolbar.

The Function Toolbar

The Function Toolbar consists of standard Windows® tools such as file open, save, and print, as well as the RamanSoft system tools SpectrumSearch, SpectrumPredict, and Real Time Monitoring.

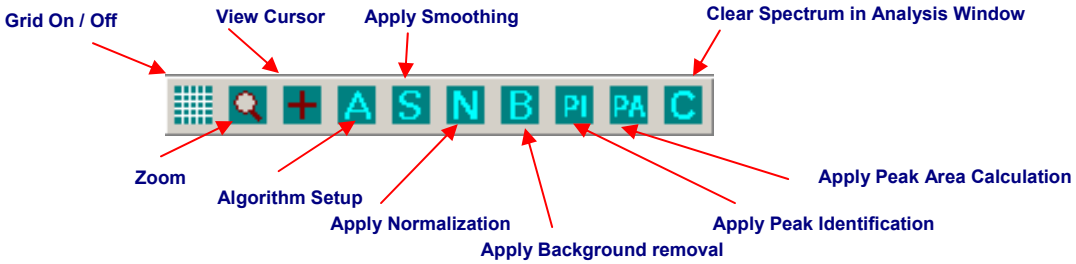


The Acquisition Window Toolbar

The Acquisition Window Toolbar is active after clicking anywhere in the Acquisition Window.

The Analysis Window Toolbar

The Analysis Window Toolbar is active after clicking anywhere in the Analysis Window.



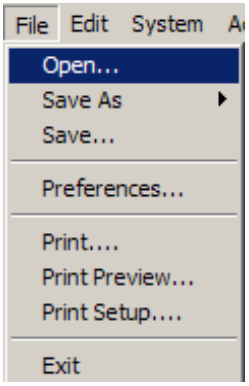
Frame Status Bar

The frame status bar which appears at the bottom of the user interface gives the x, y-coordinates of a point on a spectrum that is displayed in the Acquisition Window or in the Analysis Window. This point is identified by applying the View Cursor from the Acquisition Window Toolbar or Analysis Window Toolbar.

The x-coordinate of the spectrum point can be displayed by its pixel, wavenumber, or wavelength value. When it is displayed in wavenumber or wavelength, the units are in "cm^{-1}" and "nm", respectively. The y-axis is the intensity value in arbitrary unit. The above figure gives an example of the frame status bar when the x-axis is displayed in wavenumber.

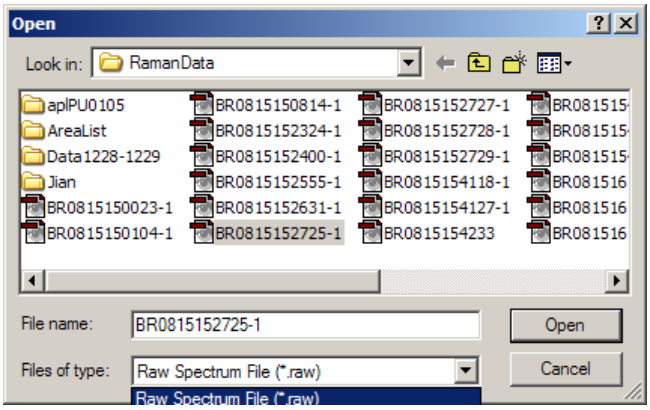
The File Menu

The File menu consists of *Open*, *Save As*, *Save*, *Preferences*, *Print*, *Print Preview*, *Print Setup*, and *Exit* sub-menu items.



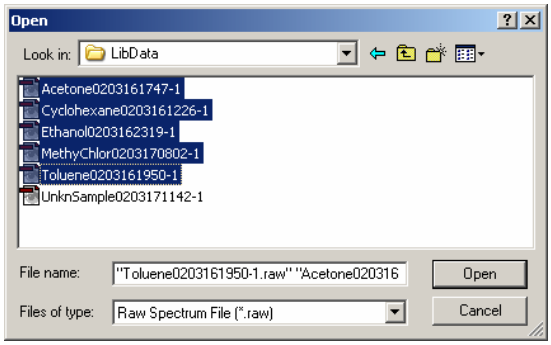
Open

This allows the user to open the raw spectrum files (*.raw) or the processed spectrum files (*.pro). Raw spectrum files and processed spectrum files, also termed collectively as “LSI formatted”, are in binary file format; they are the two fundamental file types of RamanSoft.

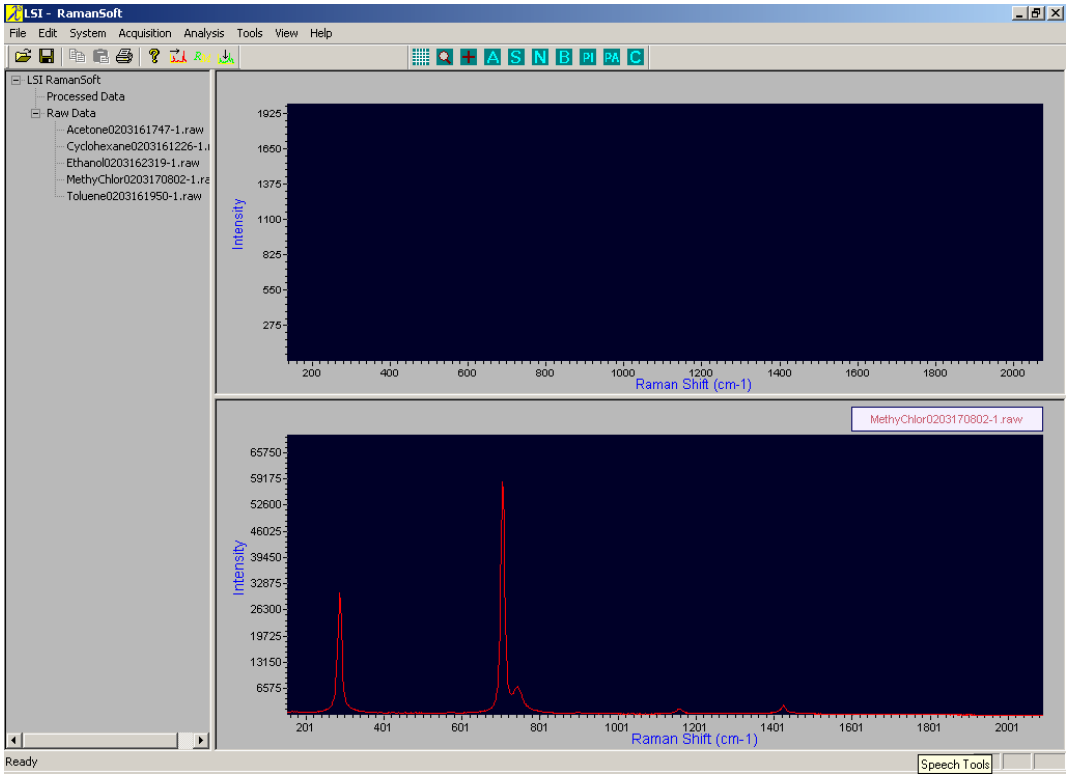


(Note that if one of the raw spectrum files in the directory was opened by double-clicking on the file name while the Adobe Photoshop program is active on the desktop, all the raw data files will be displayed in the Adobe Photoshop icons.)

The user can select and open multiple files from the same directory:

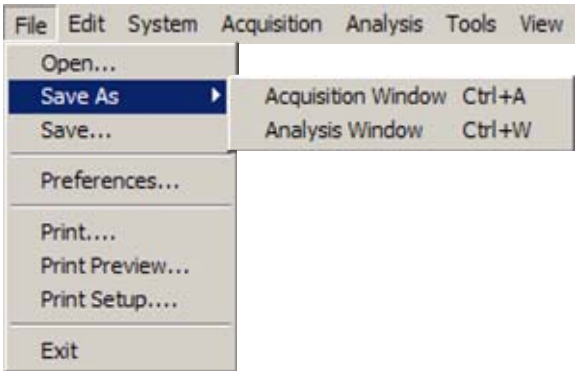


and bring the files onto the Raw Data list or the Processed Data list on the RamanSoft File View Window. One of the files is automatically displayed on the Analysis Window.



Save As

This allows the user to save a spectrum displayed in the Acquisition window as a raw spectrum file (*.raw), or save a spectrum displayed in the Analysis Window as a processed spectrum file (*.pro).



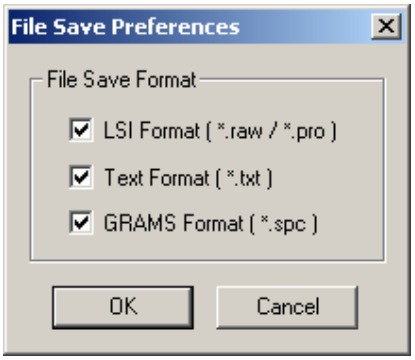
Note that a spectrum displayed in the Analysis Window can also be saved as a raw spectrum file (*.raw). This accounts for the need that when the user opens a raw spectrum file, the raw spectrum will be displayed in the Analysis Window, and the user can save the spectrum without doing any data processing but just wanting to save it into a different file name (in .raw).

Save

When the user clicks on the *Save* submenu, the raw spectrum displayed in the Acquisition Window will be saved with a system-generated filename consisting of a header (defined by the user as *File Prefix* in *Acquisition->Setup*), and a trailer (date + time, i.e., hours + minutes + seconds) generated by the system when the data was acquired. For example, "cyclohexane0203101325.raw". The saved spectrum is displayed in the Analysis Window and the filename is added onto the "Raw Data" list in the File View Window.

Preferences

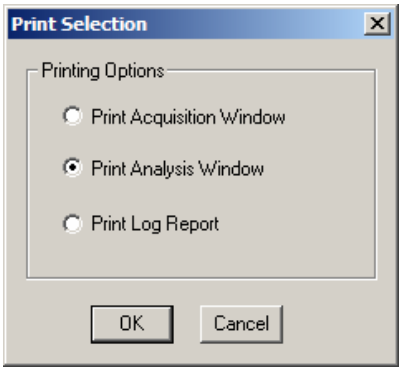
The *Preferences* submenu allows the user to choose the file save formats. By default, all the file formats, which includes LSI Format (*.raw/*.pro), Text Format (*.txt), and GRAMS Format (*.spc), are selected. The LSI Format is a binary file format, and the Text Format is a plain text format allowing the user to import the spectrum data into any program that supports this file format.



Saving files in GRAMS Format (*.spc) allows these files to be directly opened by a Thermo Galactic software such as GRAMS/AI™ 7.0, Spectral ID®, etc. We recommend that all spectra be saved in .spc so that they will be readily available for all GRAMS functionalities, however, the program allows the user to choose not to save the files in GRAMS SPC file format. To do this, just deselect the checkbox for GRAMS Format (*.spc).

Print / Print Preview

When *File->Print* or *File->Print Preview* is chosen, the Print Selection window will appear.



Print Acquisition Window allows for printing the spectrum displayed in the Acquisition Window, along with the system parameters and file information such as CCD temperature, CCD integration time, date/time, file name and path, operator name, sample name/info.

Print Analysis Window allows for printing the spectrum displayed in the Analysis Window, along with system parameters, file info, and analysis results if Peak Identification or Peak Area analysis methods were chosen.

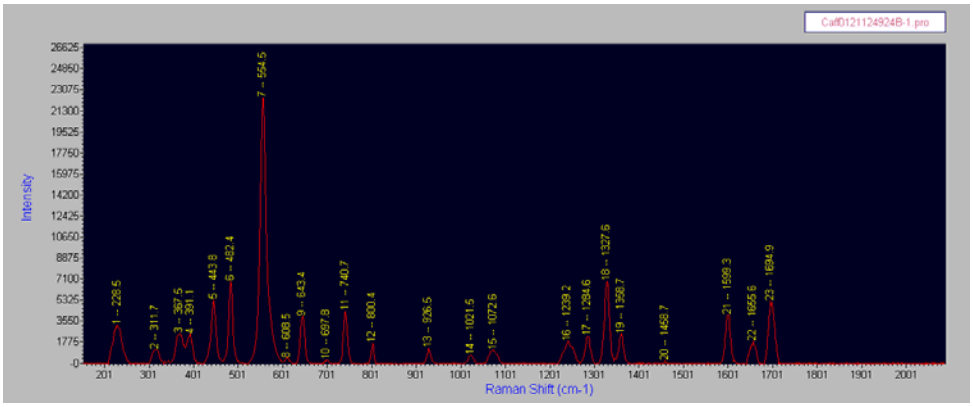
Print Log Report allows for printing the log file (a log file records all user activities for the current RamanSoft session).

If the user has chosen *File->Print Preview* and clicked *OK* in the Print Selection window, the print preview window will appear. The following figure shows the print preview of a peak-identified spectrum in the Analysis Window, along with the identified peak positions and peak intensities.

The Edit Menu

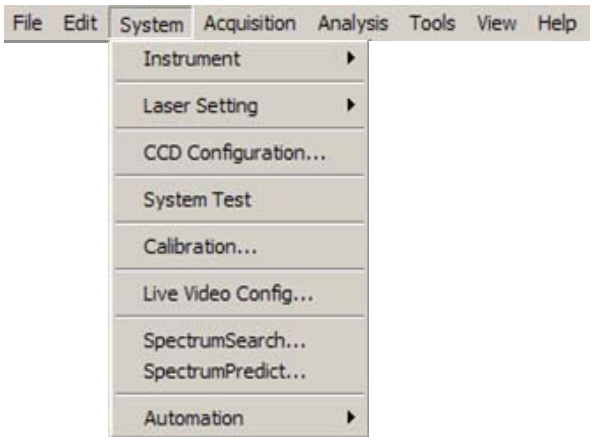
The Edit menu contains two submenu items, *Copy Acquisition View* and *Copy Analysis View*. It allows for copying the spectrum displayed in the Acquisition Window or in the Analysis Window to other applications, such as Microsoft Word, Adobe Photoshop, Microsoft PowerPoint etc.

The following figure is generated by clicking on *Edit->Copy Analysis Window* and then pasting (ctrl+V) it here in this Word document:



The System Menu

The System menu contains the following submenu items: *Instrument*, *Laser Setting*, *CCD Configuration*, *System Test*, *Calibration*, *Live Video Configuration*, *SpectrumSearch*, *SpectrumPredict*, and *Automation*.

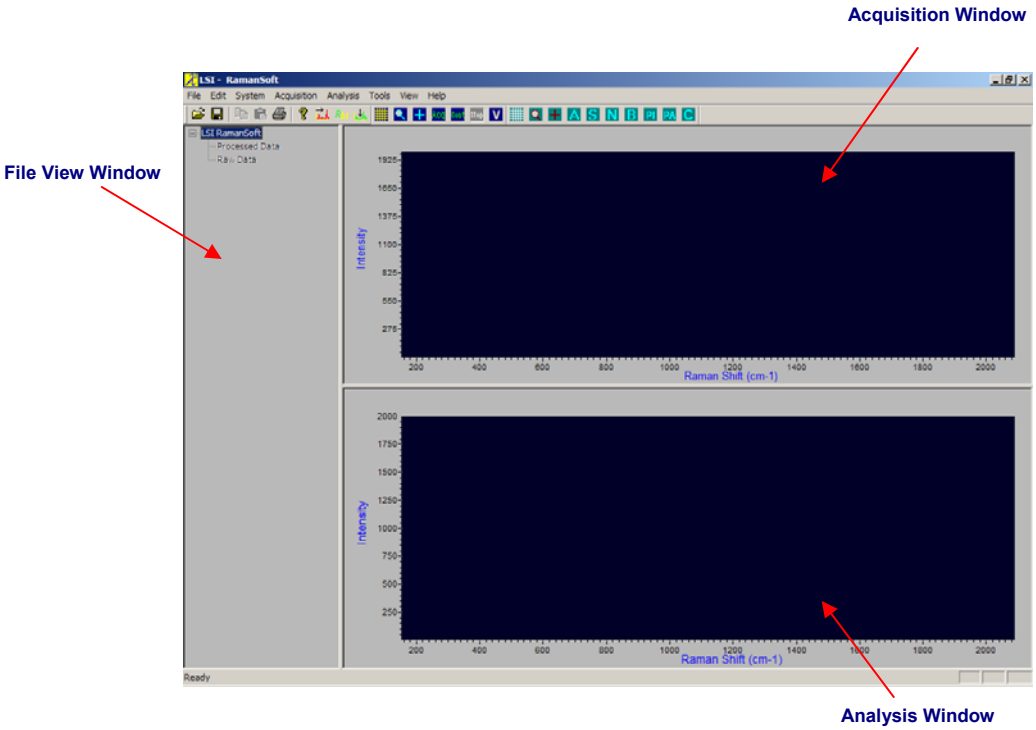


Instrument

The *graphic user interface* (GUI) for RamanSoft has two modes: the On Line mode and the Off Line mode.

On Line

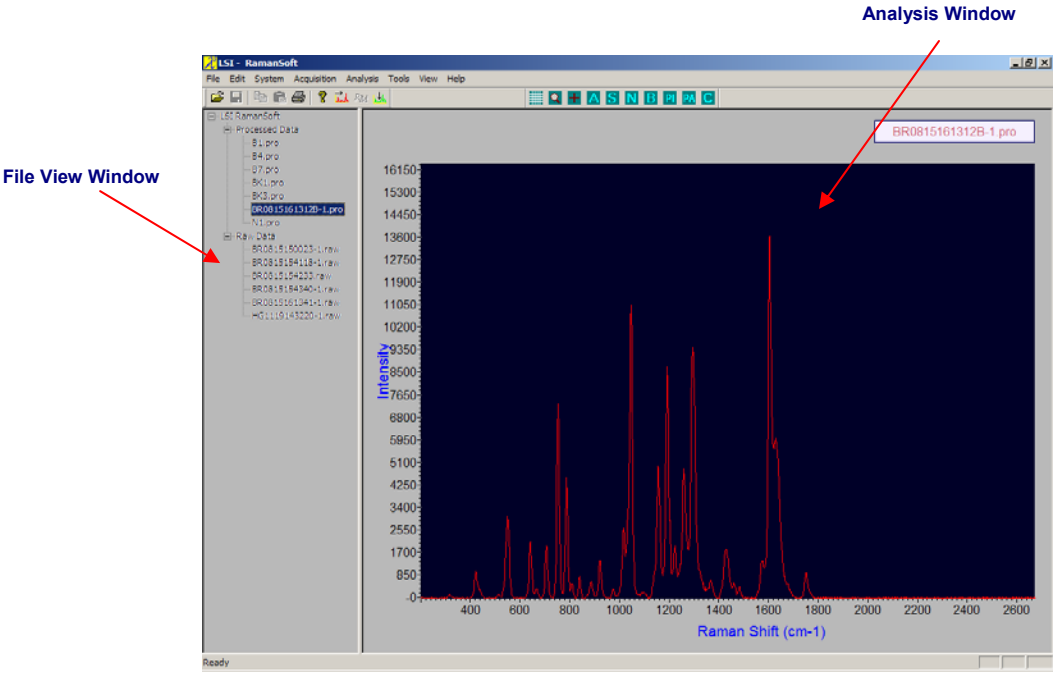
In the *On Line* mode, the acquired data is displayed in the upper Acquisition Window, and the processed data achieved through system automation is simultaneously displayed in the lower Analysis Window.



The On Line mode contains three view panels as shown above. The Acquisition Window displays the raw spectrum, the Analysis Window displays the processed spectrum when system automation is turned on, or a spectrum retrieved from the file system through *File->Open*. The File View Window displays the lists of raw and processed spectrum files in the current RamanSoft session. The ToolTip (mouse pointing to a filename) shows file info such as date, time, CCD temperature, integration time etc. The On Line mode is designed to provide the user with the options to acquire a spectrum and perform data processing simultaneously, or to perform data processing/analysis on previously acquired spectrum in the Analysis Window and in the mean time to acquire new spectrum in the Acquisition Window.

Off Line

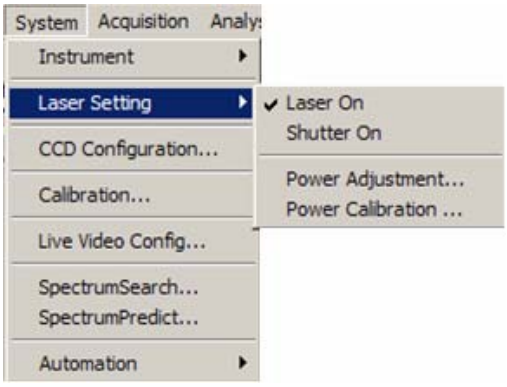
In the *Off Line* mode, only the File View Window and the Analysis Window are available; the Analysis Window occupies a larger screen area for better data display compared to that in the On Line mode.



The Analysis Window displays the spectrum retrieved through *File->Open*, and the File View Window displays the raw or processed spectrum file lists with ToolTip showing file info such as file date, time, CCD temperature, integration time etc.

Laser Setting

The *Laser Setting* submenu items provide the user with the convenience of switching laser on/off, shutter on/off, adjusting laser power, and calibrating laser power.



Laser On

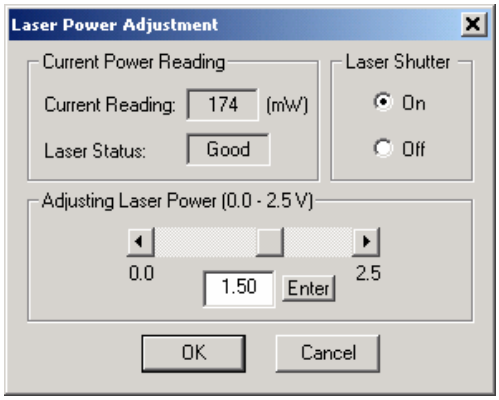
The user can turn the laser on or off by clicking on the *Laser On* submenu item, providing that the Laser Key is at the ON position on the front panel of the Dimension-P Raman unit. A check mark to the left of *Laser On* indicates that laser is turned on. The absence of this check mark (by clicking on *Laser On* again) indicates that the laser is turned off..

Shutter On

The laser shutter can be turned on/off by checking or un-checking the *Shutter On* submenu item.

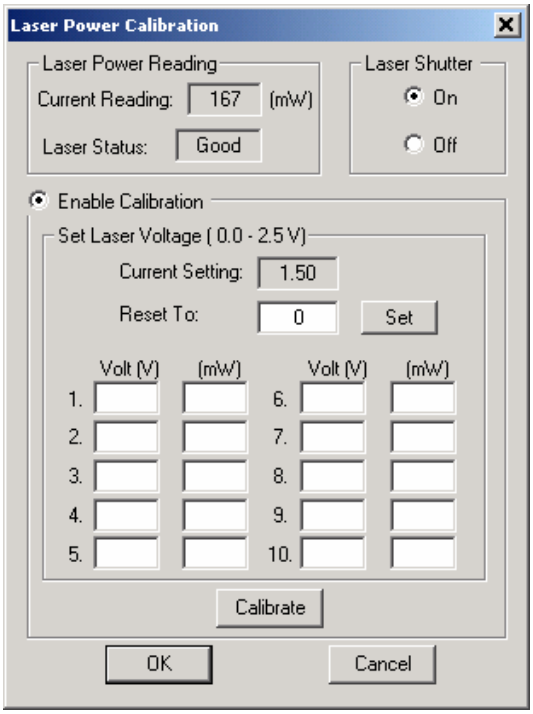
Power Adjustment

The *Power Adjustment* submenu activates the Laser Power Adjustment window. It displays the current laser power reading and the laser status, and provides switching On/Off the laser shutter. The user can adjust the laser power by using the slide bar or by entering the voltage value in the textbox under *Adjusting Laser Power* group box. Note that the user needs to make sure that the *On* radio button for *Laser Shutter* is checked while resetting the laser power.



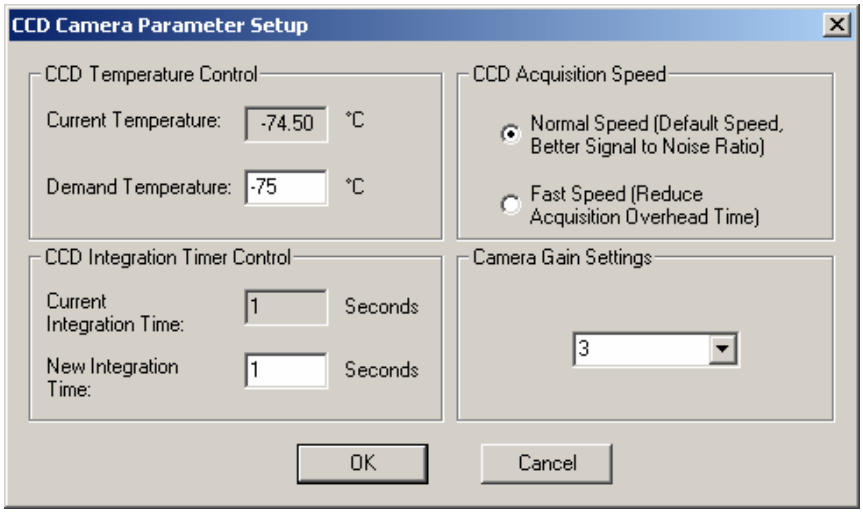
Power Calibration

The *Power Calibration* submenu item lets users calibrate the laser power versus voltage input readings by checking the *Enable Calibration* radio button. Once this radio button is checked, it enables the user to enter up to ten input voltages (V) and power (mW) value pairs. The laser voltage is entered through the *Reset To* edit box under *Set Laser Voltage* group box, and the corresponding laser power should be read directly off the power meter that the user uses. Once done, click on the *Calibrate* button and RamanSoft recalibrates the laser power as a function of the input laser voltage. Note that in order to get power readings from the power meter during the calibration process, *Laser Shutter* has to be at *On* position.



CCD Configuration

Choosing *CCD Configuration* from *System* menu activates the CCD Camera Parameter Setup window, which contains all controls for operating the CCD camera.



CCD Temperature Control

The temperature can be set as low as –80°C. But for practical purposes and for the speed of cooling we suggest -70 – -75°C as the lowest target temperature.

CCD Integration Timer Control

This permits the user to set the integration time over which a spectrum is acquired. For most samples, an integration time of 1 second provides a good spectrum.

CCD Acquisition Speed

This lets the user choose *Normal Speed* versus *Fast Speed*. At *Normal Speed*, the CCD digitization rate is set at 100 kHz, whereas at *Fast Speed*, the CCD digitization rate is set at 2 MHz. *Normal Speed* is the default choice for *CCD Acquisition Speed*; it gives better signal-to-noise ratio, but it imposes greater overhead time which is mainly caused by the CCD readout time which, in turn, is dependent on the digitization rate.

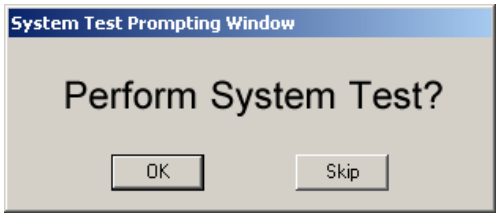
For Real Time Monitoring applications this overhead time will affect fast process monitoring. An integration time of 50 milliseconds and above at 100 kHz yields acceptable linearity (for e.g., 200 milliseconds integration time yields 4 times the intensity of that of 50 milliseconds). Thus for high speed monitoring we recommend the *Fast Speed.* While the *Fast Speed* mode does not give the maximal signal-to-noise characteristics as that of the *Normal Speed* mode, it reduces the overhead time and makes monitoring at acquisition times below 50 milliseconds more practical. At 2 MHz, linearity can be achieved down to 10 milliseconds. The user is encouraged to see more details on “Acquisition Speed” in Chapter 8, the Real Time Monitoring Module.

Camera Gain Settings

The CCD camera gain settings can be set from 1 to 3, with 3 being the highest gain. The readout intensity is reduced by approximately 2 fold for each decrement in gain. By default, gain setting 3 is used. The user may wish to use a lower gain when at a given integration time when the intensity values are close to or above the maximum CCD readout value (i.e., 65,536, or 2^{16} for a raw spectrum), especially for samples with strong fluorescence background.

System Test

Every time RamanSoft is started the user is prompted with the System Test Window. In addition, the *System Test* menu item provides the user an opportunity to perform a system test at any time during a RamanSoft session. It is recommended that the user perform system test daily to make sure that the instrument remains calibrated so that any data acquired are accurate.



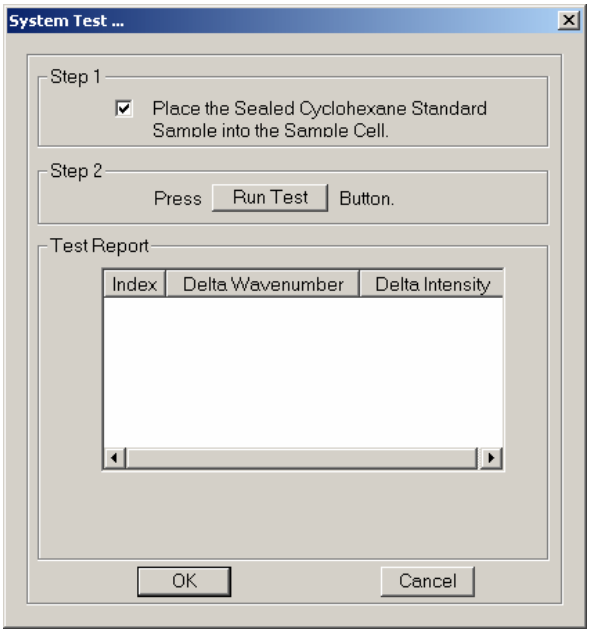
To Skip System Test

Press *Skip*, the three-window panel RamanSoft GUI then appears and the user is ready to use to system assuming that the system stays calibrated..

To Perform System Test

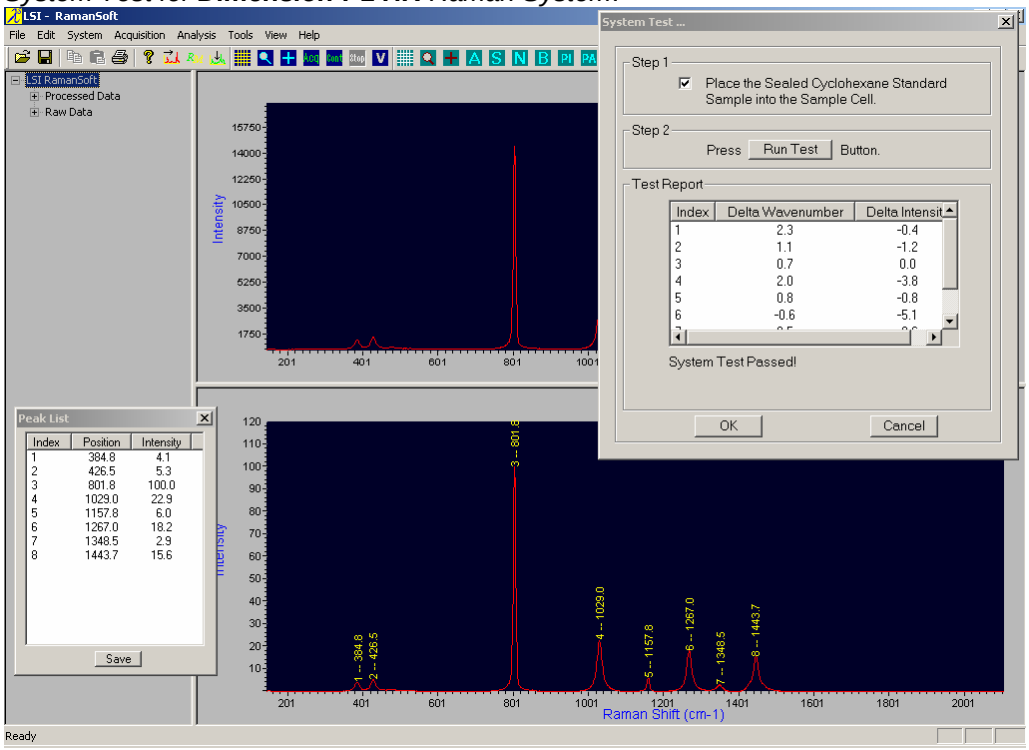
1. Press *OK.* The System Test… window appears.

2. Put the sealed cyclohexane standard sample into the internal sample cell or External Sampling Module, and check *Step 1* checkbox.

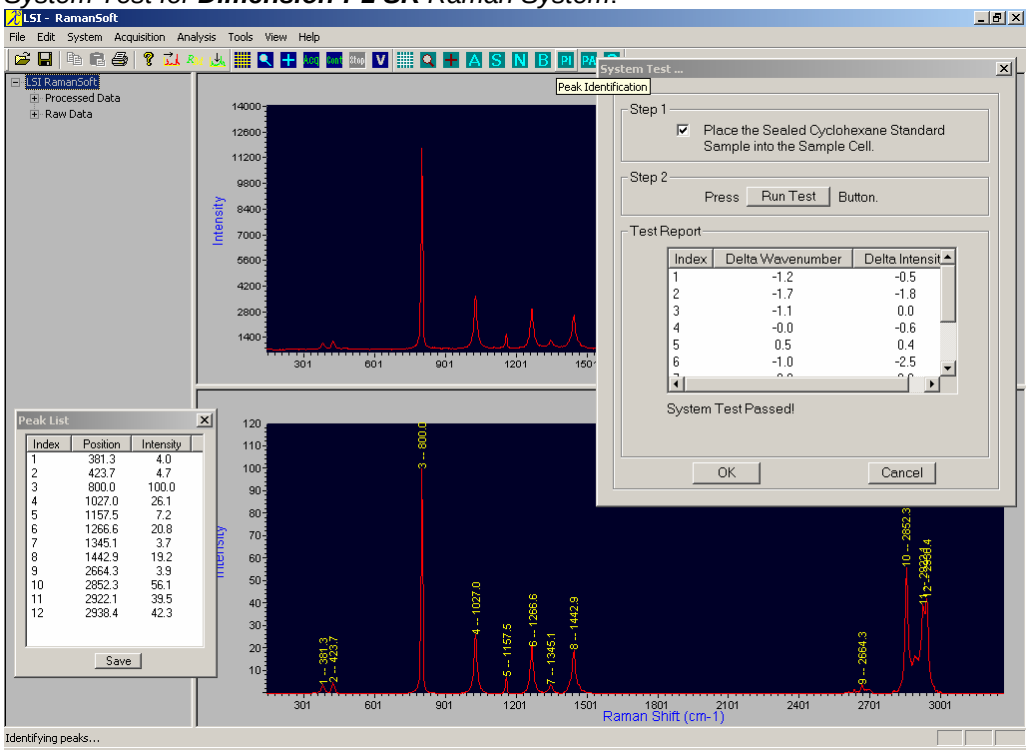


3. Press *Run Test* button in Step 2 group box. The system takes the cyclohexane spectrum, identifies the cyclohexane peaks, and updates the System Test window with test results.

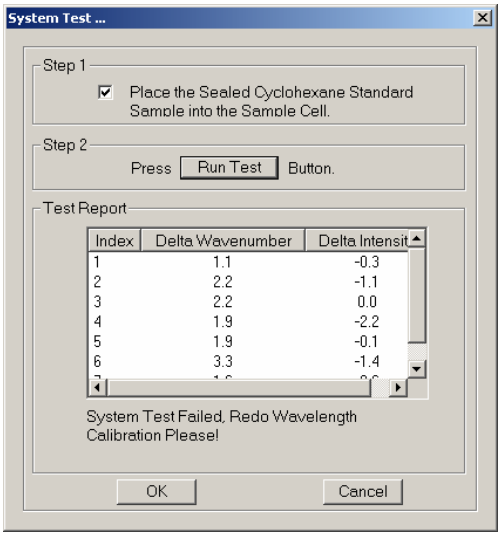
*System Test for **Dimension-P1 HR** Raman System.*



*System Test for **Dimension-P1 SR** Raman System.*

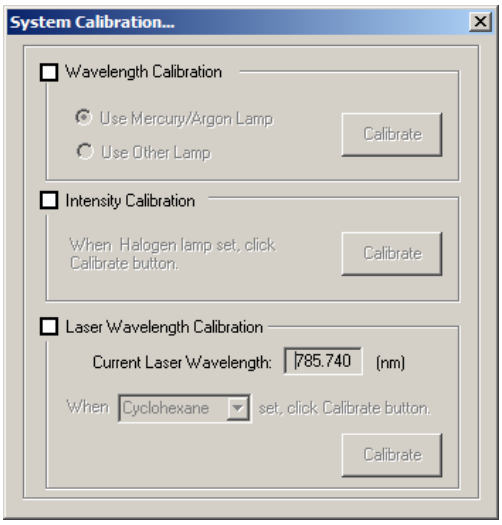


4. View *Test Report*. The program uses 8 cyclohexane peaks for the system test of Dimension-P1 High Resolution (HR) models and the first 10 cyclohexane peaks for the system test of Dimension-P1 Standard Resolution (SR) models, respectively, as labeled in the above figures. It compares the position and intensity of each peak to those of the cyclohexane standard spectrum stored in the system. The differences in the peak position and peak intensity (*Delta Wavenumber*, *Delta Intensity*, the second and third column in the *Test Report* panel) are calculated for each of the seven peaks. If any of the *Delta Wavenumber* values is larger than the Wavenumber Tolerance set by the system, the system test is judged as "Failed" and the user is suggested to perform the Wavelength Calibration and Laser Wavelength Calibration, the two of the three calibration procedures that ensure correct x-positions of the peaks in the System Calibration routine (please refer to section "Calibration" for details on System Calibration) (see below figure). Likewise, if any of the *Delta Intensity* values is larger than the Intensity Tolerance set by the system, the system test is also judged "Failed" and the user is suggested to perform the Intensity Calibration, the procedure that ensures the correct intensity values of the peaks. If both failed, then the user is suggested to perform all three system calibration routines.

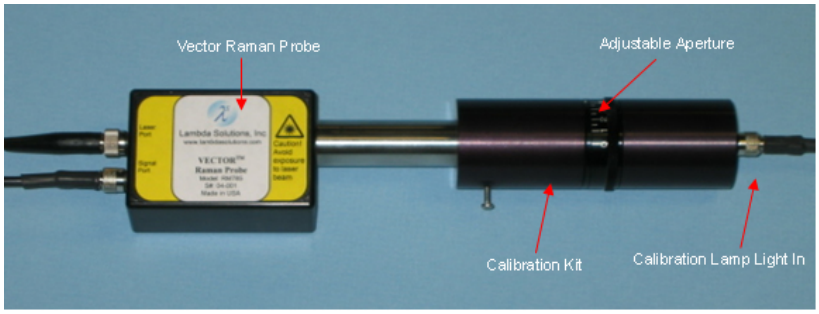


Calibration

Choosing *Calibration* from the *System* menu brings up the System Calibration window, which outlines the three calibration steps.



System calibration is performed with the LSI Calibration Kit. To do this, the user needs to insert the Vector Raman Probe into the LSI Calibration Kit as shown below, turn the adjustable aperture to allow highest (but not saturated) signal intensity.



Wavelength Calibration

The user can choose a Mercury/Argon lamp to perform auto wavelength calibration or any other standard lamp to perform manual wavelength calibration with the Vector Raman Probe and the Calibration Kit.

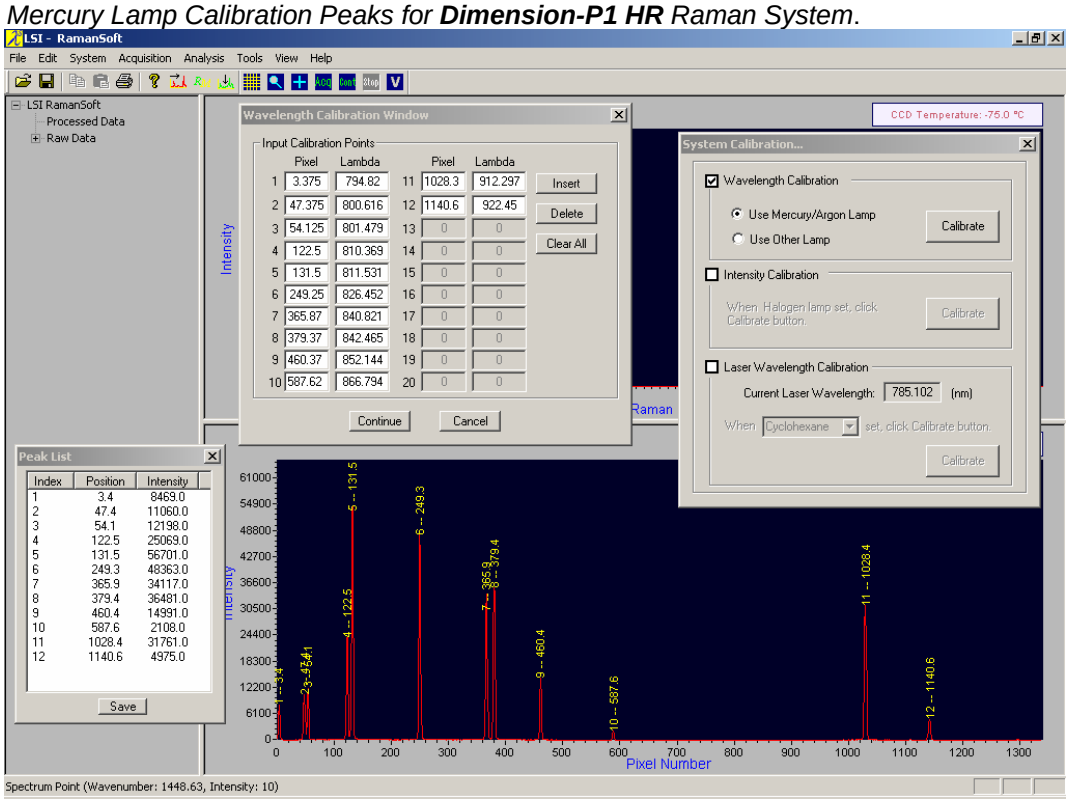
Auto Wavelength Calibration

1. From the main menu, choose *System->CCD Configuration* to set the *New Integration Time* to be 1 second.

2. Check *Wavelength Calibration* checkbox in the System Calibration window, and check *Use Mercury/Argon Lamp* radio button. Press the *Calibrate* button.

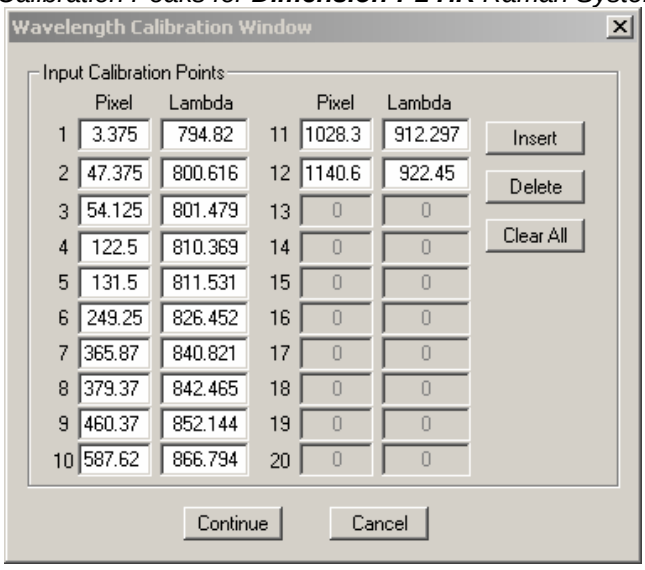
3. The program starts the acquisition of the mercury/argon lamp spectrum, and automatically identifies the mercury lamp peaks used for auto wavelength calibration.

*Mercury Lamp Calibration Peaks for **Dimension-P1 HR** Raman System.*



For Dimension-P1 high resolution (HR) models, the program uses 12 standard Mercury/Argon peaks (as labeled in the above figure and tabulated in the Wavelength Calibration Window below) to perform the wavelength calibration (see Appendix II for a full-page view of the above figure where the 12 standard peaks can be clearly discerned, and a table for the mercury/argon lamp peak wavelengths).

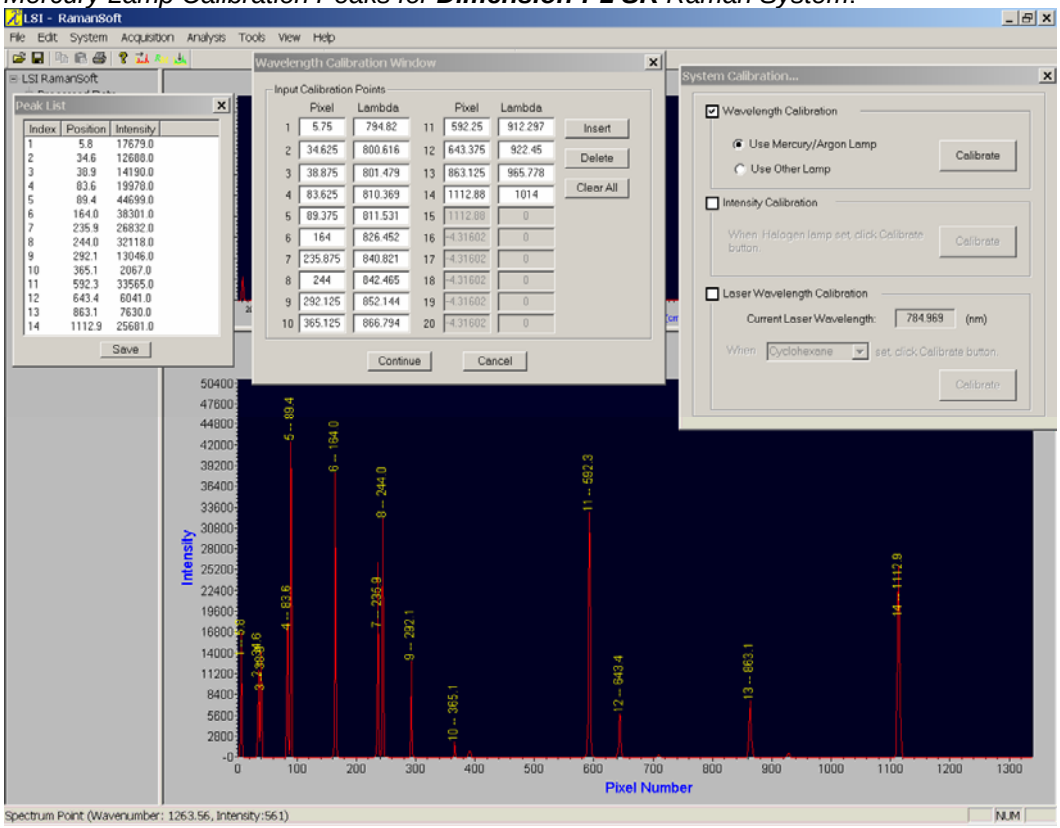
*Mercury Lamp Calibration Peaks for **Dimension-P1 HR** Raman System.*



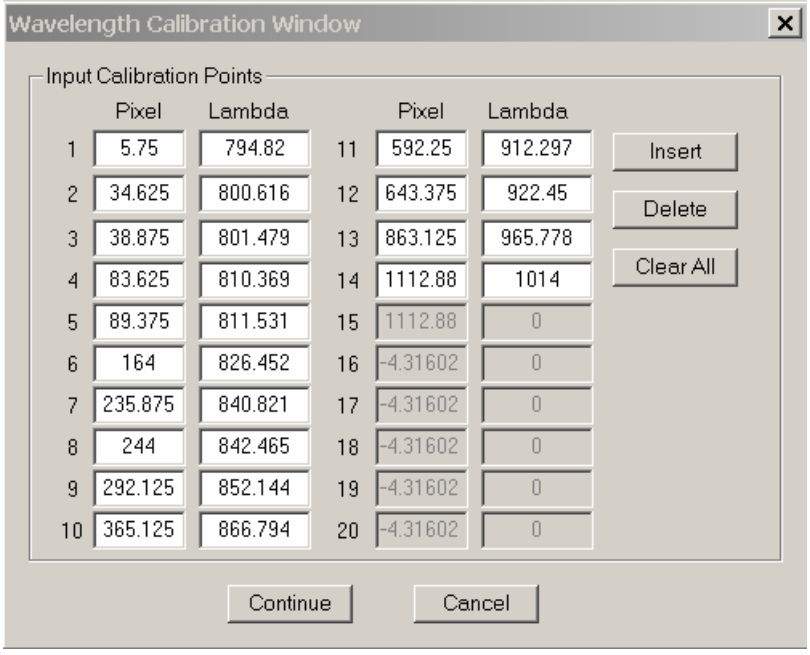
For Dimension-P1 standard resolution (SR) models, the program uses 14 standard Mercury/Argon peaks (as labeled in the below figure and tabulated in the below Wavelength Calibration Window) to perform the wavelength calibration (also see Appendix II for a full-

page view of the below figure where the 14 standard peaks can be clearly discerned, and a table for the mercury/argon lamp peak wavelengths).

*Mercury Lamp Calibration Peaks for **Dimension-P1 SR** Raman System.*



*Mercury Lamp Calibration Peaks for **Dimension-P1 SR** Raman System.*



The pixel position of each peak as identified by the program is displayed in the *Pixel* column in the Wavelength Calibration Window. To increase the calibration accuracy, the program employs a cubic spline interpolation procedure to improve peak symmetry and thus obtain true peak positions. The fractional pixel number in the *Pixel* column results from this interpolation procedure. The corresponding standard wavelengths (in nm) of the mercury lamp peaks are displayed in the *Lambda* column. If all the peaks are correctly identified (after user's verification), the user can press *Continue* and the system automatically finishes the wavelength calibration.

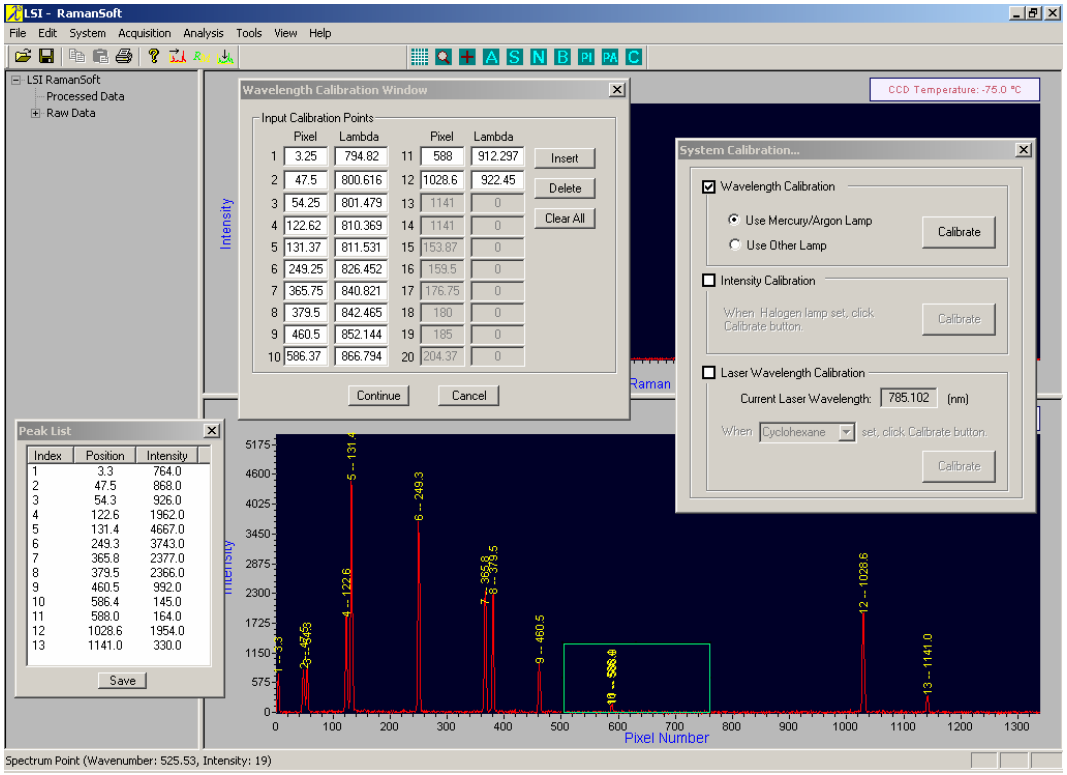
Note: Note that the pixel numbers in the above figure and in Appendix II serve as a guide. The exact pixel numbers will depend on a specific instrument calibration.

We recommend that the user change the aperture setting on the Calibration Kit, press the *Clear All* button on the tabulation screen, and repeat the auto wavelength calibration procedure. Statistics indicates that auto wavelength calibration at the top three aperture settings yields identical and correct results for >98% of the time.

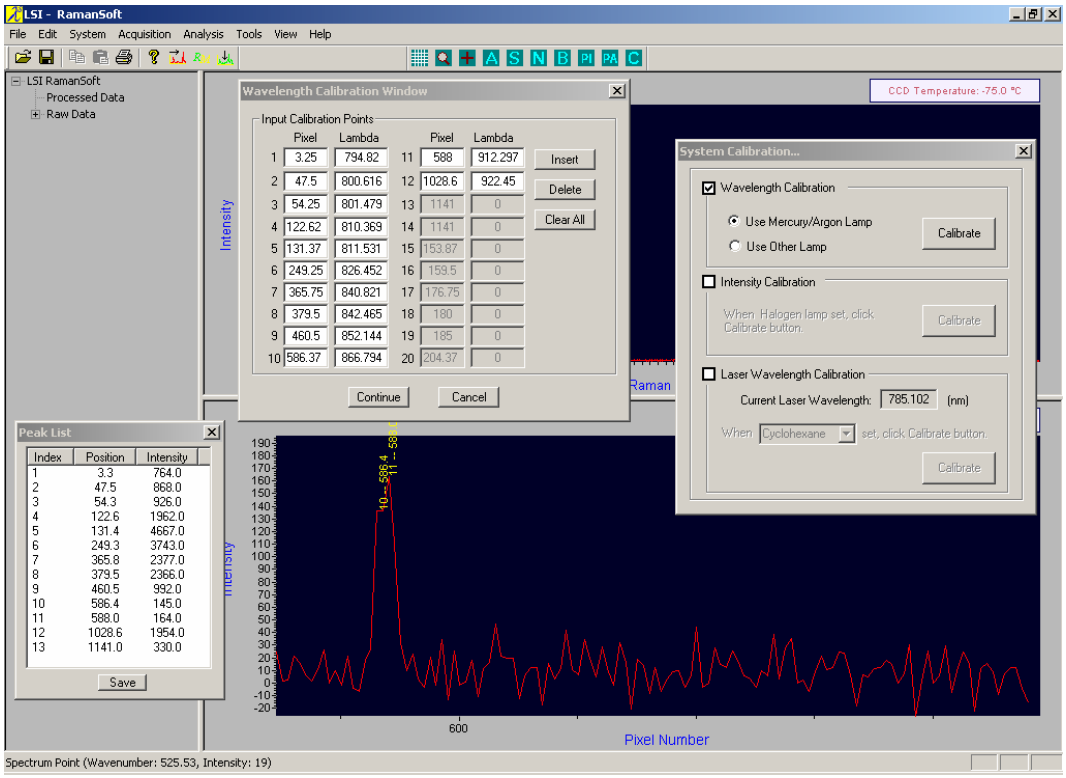
Manual Editing During Auto Wavelength Calibration

If the program identifies wrong or extra peaks, i.e., it misses or assigns extra pixel numbers in the *Pixel* column, the user can use the *Insert* or *Delete* button to insert/delete the correct pixel numbers for those missing/extra peaks (use View Cursor  in the Analysis Window Toolbar to manually identify those peaks first), and then press *Continue* to finish the wavelength calibration.

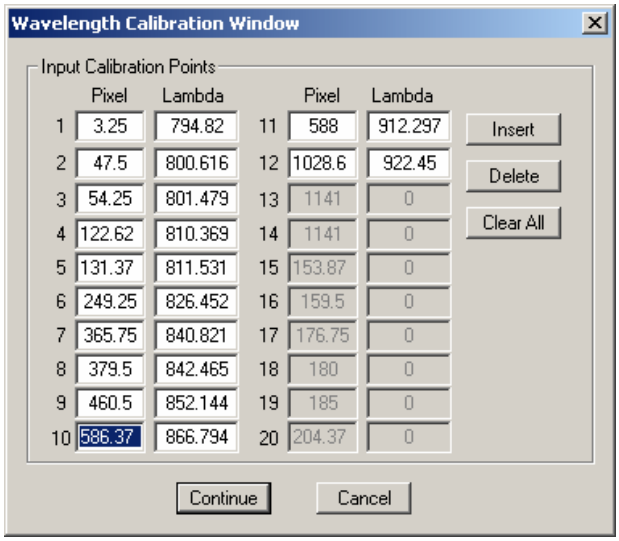
For example, the following figure shows a scenario where an extra peak (Peak 10 or 11) has been assigned to the Pixel column.



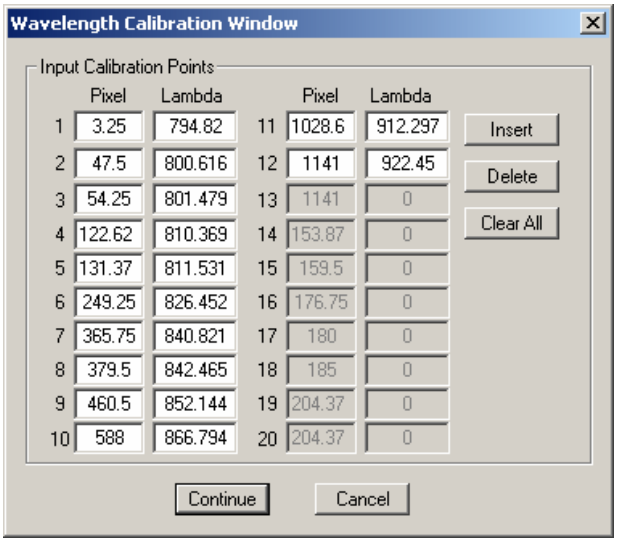
By zooming in to the peak 10 and peak 11 region as shown in the below figure, we can identify that the extra peak is peak 10.



Highlight or hold the left mouse button down in the Peak 10 Pixel column as shown below,



and click on *Delete* button, the extra peak 10 is then deleted from the Pixel column. The next pixel value, 588, which is the correct pixel corresponding to wavelength 866.794 nm, is shifted up.



The user can then press the *Continue* button, and the system automatically finishes the wavelength calibration.

If a peak is not identified by the program and thus is not displayed in the Pixel column, the user can use the same procedure as described above to add the missed pixel number in. First, highlight or hold the left mouse button down in the peak textbox right after the missing peak, click on the *Insert* button, a new textbox with the same pixel number will then be inserted. Type in the right peak pixel number of the missing peak in the textbox. Once the user has confirmed all the peak readings, the user can then click on the *Continue* button, and the system will automatically finish the wavelength calibration.

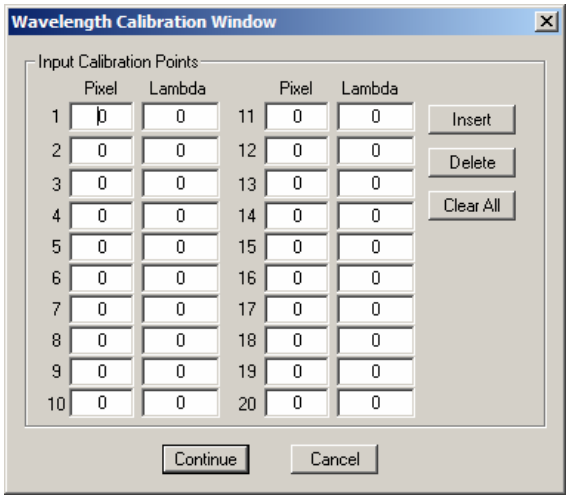
Manual Wavelength Calibration

1. From the main menu, click on *System->CCD Configuration* to set *CCD Integration Time* to be 1 second.

2. Check *Wavelength Calibration* checkbox in the System Calibration window, choose *Use Other Lamp*. Press the *Calibrate* button.

3. RamanSoft will start acquiring the lamp spectrum and performing peak identification on the spectrum in the Analysis Window.

4. A dialog window will appear as shown below, which allows the user to manually enter the pixel numbers and the corresponding wavelength (in nm) values of the peaks that the user chooses to use for the calibration. The maximum number of peaks that can be used for the manual wavelength calibration is 20.



5. Once finished, the user can click on the *Continue* button, and RamanSoft will perform the calibration accordingly.

Intensity Calibration

The intensity calibration can be performed with the LSI Calibration Kit and Vector Raman Probe according to the following steps:

1. Check the *Intensity Calibration* checkbox in the System Calibration window.

2. Turn on a NIST traceable blackbody radiator (e.g., LS-1-Cal calibration lamp from Ocean Optics) for 20 to 30 minutes to allow it to reach equilibrium.

3. Attach the calibration lamp and the Vector Raman Probe to the LSI Calibration Kit.

4. From the main menu, click on *System->CCD Configuration* to set *CCD Integration Time* to be 1 sec.

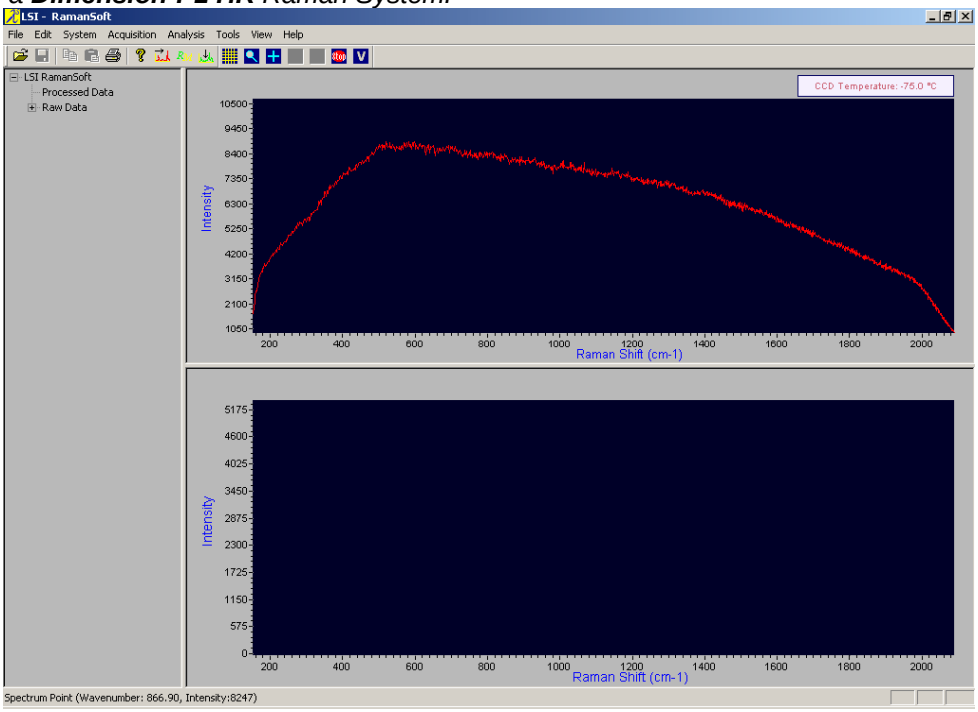
5. Click on *Acquire->Continuous Acquire* (or click 

on the Acquisition Window Toolbar) so that the calibration lamp spectrum is displayed continuously in the Acquisition Window.

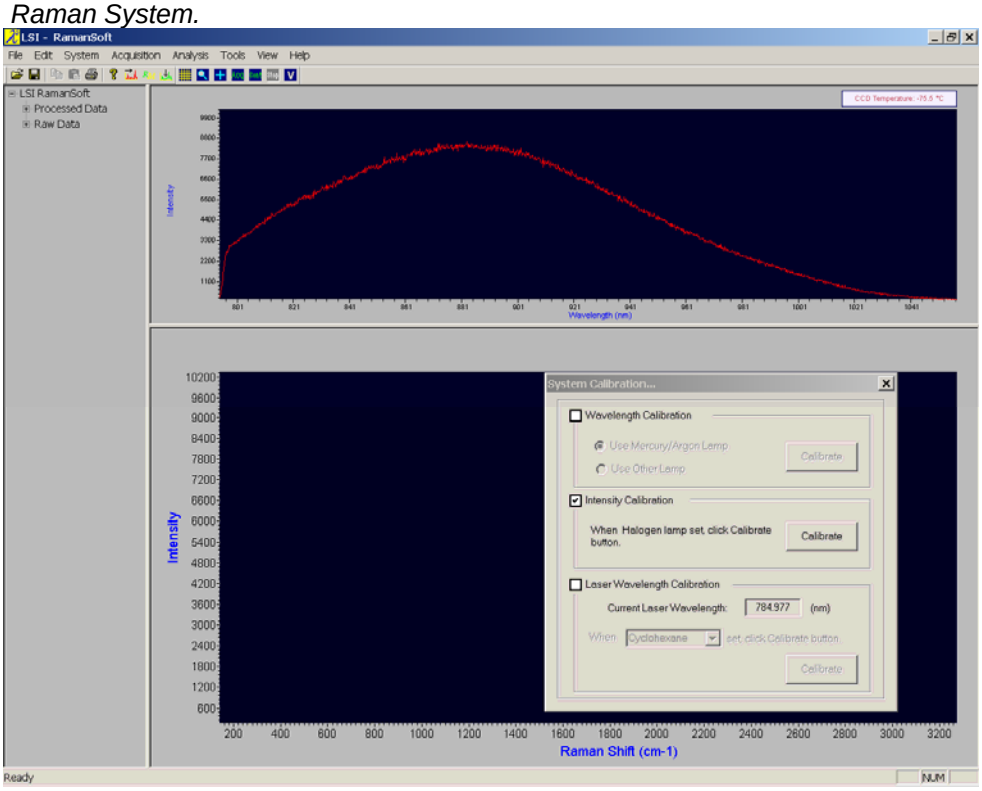
6. Adjust the aperture of the Calibration Kit to obtain the optimum Intensity level for the calibration lamp spectrum (maximum intensity without saturation). Once the calibration lamp spectrum reaches the optimum Intensity level, click on *Acquisition->Stop Acquire* or press 

to stop the continuous acquisition.

*Continuous acquisition of the LS-1-Cal (Ocean Optics, Inc.) calibration lamp spectrum on a **Dimension-P1 HR** Raman System.*



*The LS-1-Cal (Ocean Optics, Inc.) calibration lamp spectrum on a **Dimension-P1 SR** Raman System.*



7. Click on the *Calibrate* button in the System Calibration window, RamanSoft acquires the calibration lamp spectrum and performs the Intensity calibration automatically.

Laser Wavelength Calibration

Laser wavelength calibration can be performed in auto mode (with user validation) using the sealed cyclohexane standard sample or in manual mode using any other standard sample that the user chooses.

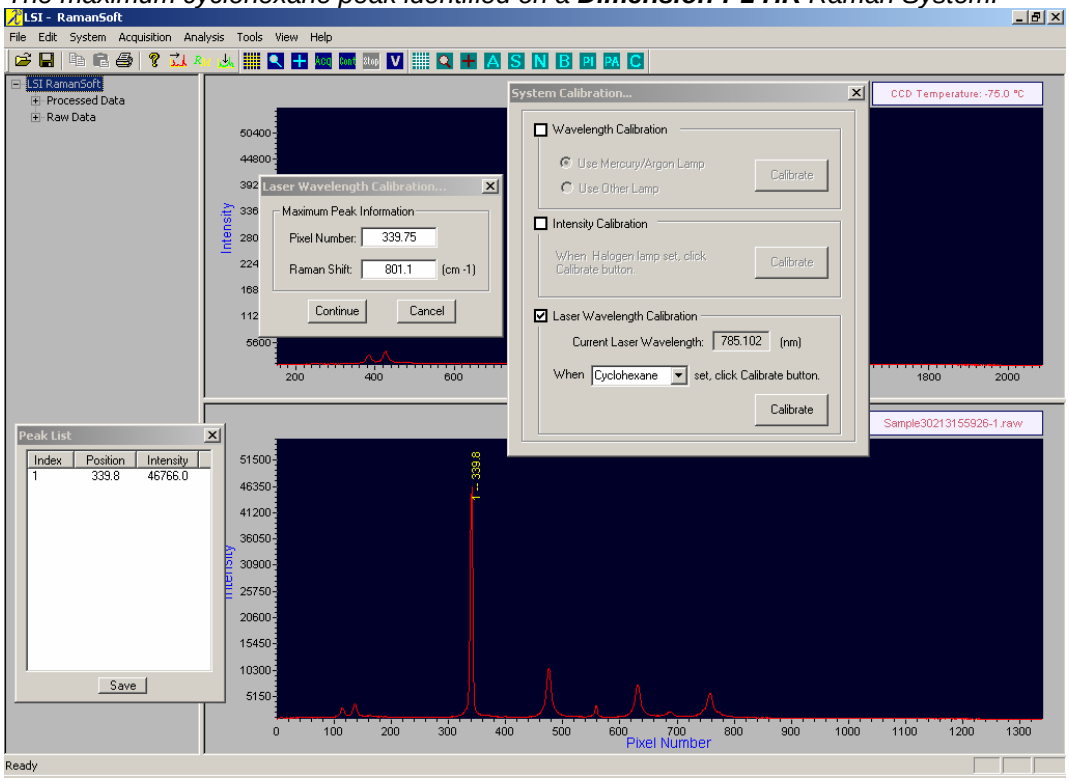
Auto Laser Wavelength Calibration

1. Check the *Laser Wavelength Calibration* checkbox in the System Calibration window.

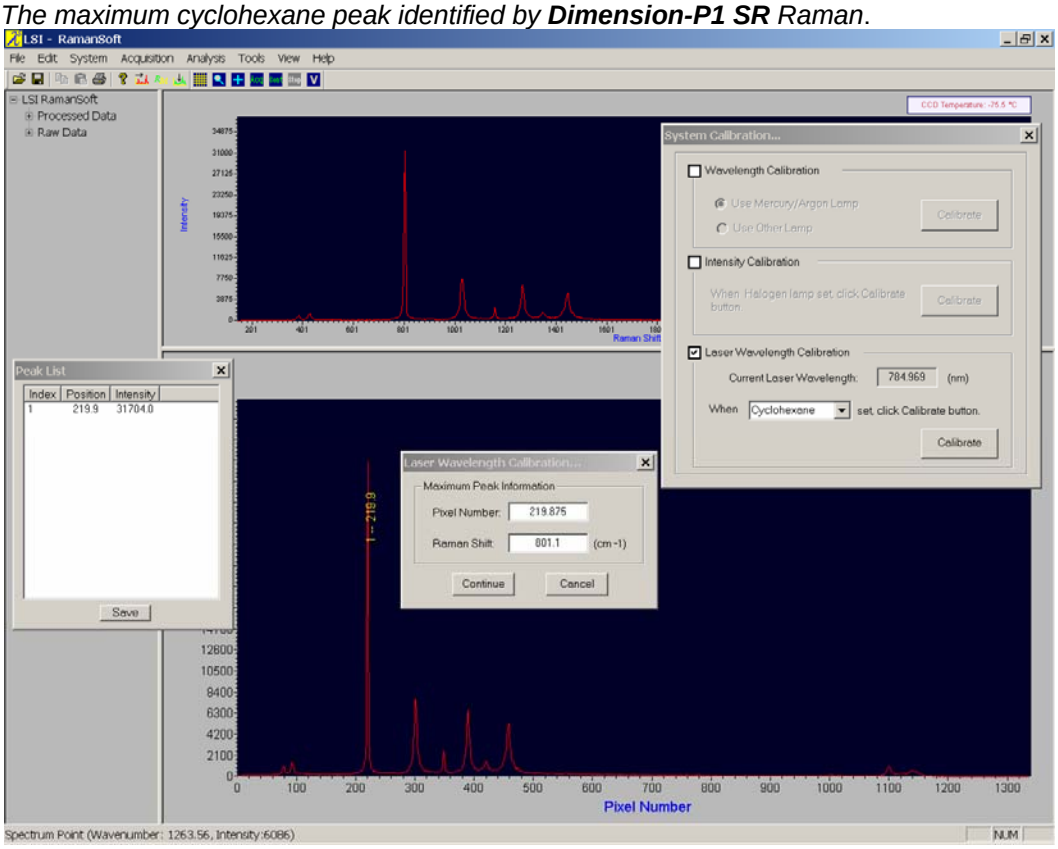
2. Put the sealed cyclohexane standard sample in the sample holder. Click on *System->CCD Configuration* to set the *New Integration Time* to be 1 sec.

3. Click on the *Calibrate* button. RamanSoft starts to acquire the cyclohexane spectrum and identifies the pixel position of the maximum peak, which are shown below and also in full-page view in Appendix III.

*The maximum cyclohexane peak identified on a **Dimension-P1 HR** Raman System.*

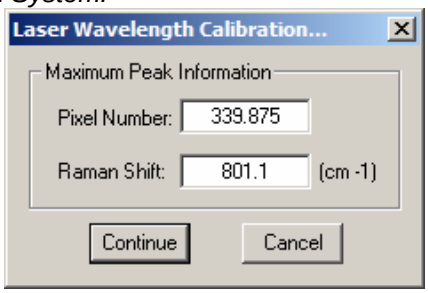


*The maximum cyclohexane peak identified by **Dimension-P1 SR** Raman.*

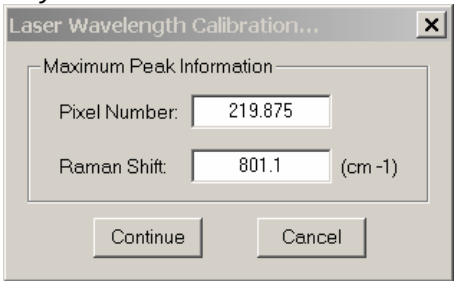


4. The Laser Wavelength Calibration… window appears displaying the identified pixel number and the corresponding Raman shift of the maximum peak of cyclohexane (801.1 cm^{-1}). Similar with the Wavelength Calibration, the program utilizes cubic spline interpolation procedure to improve calibration accuracy, and thus the pixel number might be fractional.

*The pixel position and the Raman Shift of the maximum cyclohexane peak identified on a **Dimension-P1 HR** Raman System.*



*The pixel position and the Raman Shift of the maximum cyclohexane peak identified on a **Dimension-P1 SR** Raman System.*



5. Click on *Continue* button once the user has confirmed the *Pixel Number* reading of the maximum cyclohexane peak, RamanSoft will then perform the laser wavelength calibration.

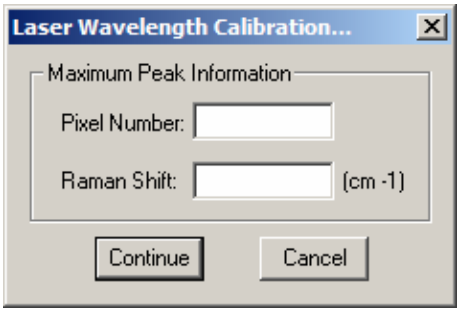
Manual Laser Wavelength Calibration

1. Put the user-chosen standard sample into the sample holder. Click on *System->CCD Configuration* to set the *New Integration Time* to be 1 sec.

2. Check the *Laser Wavelength Calibration* checkbox in the System Calibration window. Choose *Other Sample* in the pull-down selection menu.

3. Click on the *Calibrate* button. RamanSoft starts to acquire standard sample spectrum and identifies the maximum peak position.

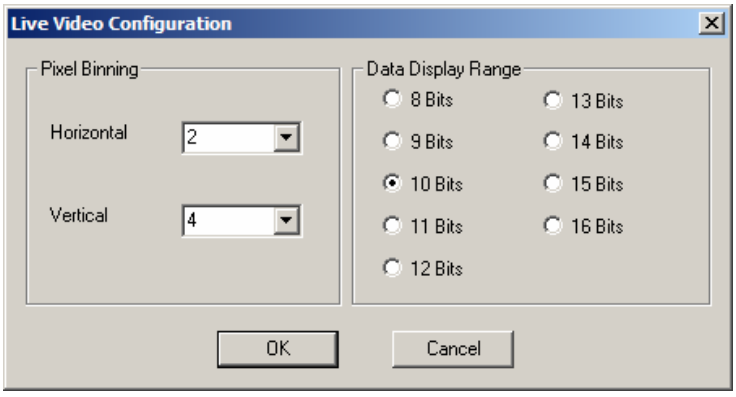
4. The Laser Wavelength Calibration… window appears. Both the *Pixel Number* and the corresponding *Raman Shift* value must be entered manually.



The calibrated wavelength, wavenumber, and efficiency correction through intensity calibration are saved in C:\LSI\RamanSoft\Calibration. The file is named using the system date and time (including year, e.g., 20050228084333). This is a text file with .cal extension.

In addition, spectrum data for wavelength calibration (e.g., mercury lamp spectrum), intensity calibration (e.g., halogen calibration lamp spectrum), and laser wavelength calibration (e.g., cyclohexane spectrum) are saved in C:\LSI\RamanData\CalibrationData. These are files in LSI .raw format and also text format with filenames such as WL0228084148.raw for wavelength calibration data, INT0223154319.raw for intensity calibration data, and LW1223154320.raw for laser wavelength calibration data. The numbers stands for the system date and time.

Live Video Configuration



The *Live Video Configuration* menu provides the settings to configure for acquiring and displaying a CCD image. This is an essential tool for an system engineer to adjust alignment of fiber optics in the Raman spectrometer system.

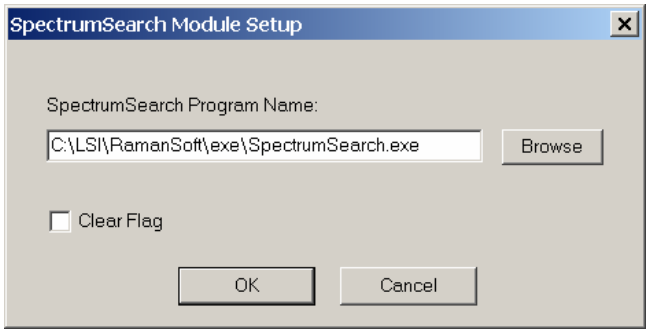
The *Pixel Binning* group box provides an option for the user to set the horizontal or vertical binning factors for CCD imaging acquisition and display. The *Data Display Range* has from 8 Bits to 16 Bits data format for user to choose. The higher the bits, the better the image contrast, but sensitivity is reduced and the acquisition will be slower.

To use the live video mode, put a sample of interest into the sample cell, set CCD integration time and gain setting in *System->CCD Configuration*. Activate the live video mode by clicking on  on the Acquisition Window Toolbar. The video image for the spectrum is then displayed in the Acquisition Window. Clicking on  the second time to quit the live video image mode.

Note: The Live Video mode is mainly for field engineers. Users are not recommended to use this functionality.

SpectrumSearch

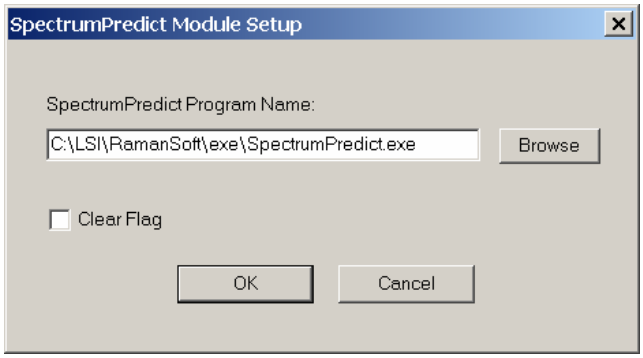
The *SpectrumSearch* submenu allows the user to locate the executable SpectrumSearch.exe from your computer hard disk for performing SpectrumSearch application (see Chapter 6 for details on the SpectrumSearch Module). This is used only when the SpectrumSearch module has been moved from its default location at C:\LSI\RamanSoft\exe when RamanSoft is first installed.



The *Clear Flag* check box allows the user to clear any errors before re-starting the SpectrumSearch module in the event the module crashes due to a run time error or any other error source.

SpectrumPredict

The *SpectrumPredict* submenu allows the user to locate the executable SpectrumPredict.exe from your computer hard disk for performing SpectrumPredict application (see Chapter 7 for details on the SpectrumPredict Module). This is used only when the SpectrumSearch module has been moved from its default location at C:\LSI\RamanSoft\exe when RamanSoft is first installed.



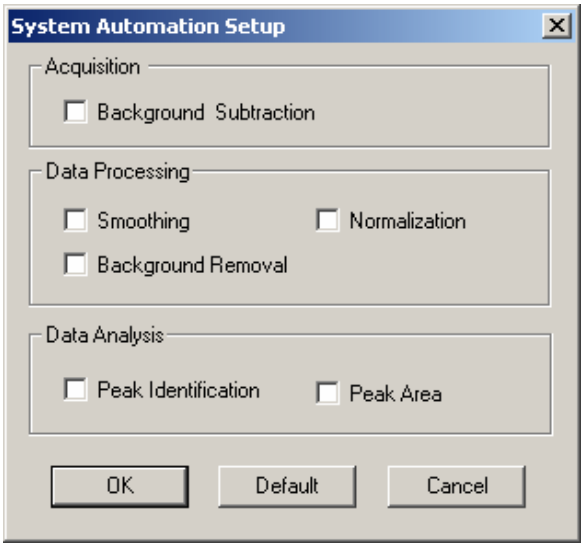
The *Clear Flag* check box allows the user to clear any errors before re-starting the SpectrumSearch module in the event the module crashes due to a run time error or any other error source.

Automation

The automation function of RamanSoft allows the user to apply data acquisition/ processing/ analysis methods during the spectrum acquisition process.

Automation Setup

Clicking on *Automation->Setup* activates the System Automation Setup window, where the user can activate various data acquisition / processing / analysis methods.



The *Background Subtraction* function allows the user to subtract a background spectrum from the spectrum data during acquisition. Configuration of a specific *Background Subtraction* method can be achieved through *Acquisition->Setup*. Refer to the subsections “Setup” and “Background” under “The Acquisition Menu” section in this chapter for details.

The *Smoothing* allows users to apply a smoothing algorithm to the acquired spectrum. Configuration of which smoothing algorithm to apply (Adjacent Average, Savitzky-Golay, FFT Filter) can be achieved through the *Analysis->Algorithm Setup->Smoothing* tab page. Refer to the subsection “Algorithm Setup” under “The Analysis Menu” section in this chapter for details.

Normalization allows users to apply a normalization algorithm to the acquired spectrum. Configuration of which normalization algorithm to apply (normalize to laser power, to maximum peak intensity in full spectrum range or a user defined range, or to the intensity at a specific spectrum point) can be achieved through the *Analysis->Algorithm Setup->Normalization* tab page. Refer to the subsection “Algorithm Setup” under “The Analysis Menu” section in this chapter for details.

Background Removal allows the user to apply a background removal algorithm to the acquired spectrum. Configuration of which algorithm to apply (automatic, semi-automatic, manual) can be achieved through the *Analysis->Algorithm Setup->Background Removal* tab page. Refer to the subsection “Algorithm Setup” under “The Analysis Menu” section in this chapter for details.

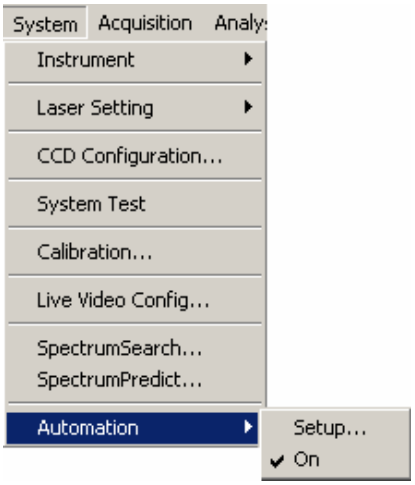
Peak Identification allows the user to perform peak labeling to the acquired spectrum. Configure which algorithms to apply (default, customer chosen peaks) from the *Analysis->Algorithm Setup->Peak Identification* tab page. Refer to the subsection “Algorithm Setup” under “The Analysis Menu” section in this chapter for details.

Peak Area allows the user to perform peak area calculation to the acquired spectrum. To automate peak area calculation, the user needs to configure “Auto” mode in the *Analysis->Algorithm Setup->Peak Area* tab page. Refer to the subsection “Algorithm Setup” under “The Analysis Menu” section in this chapter for details.

Auto File saving

If there is one or more than one data acquisition/ processing/ analysis methods checked in the System Automation Setup window, the system automation is turned on and both the raw and the processed spectra are automatically saved according to the *Path* and *File Prefix* fields set up in the Acquisition Setup window.

If no data acquisition/ processing/ analysis methods is checked in the System Automation Setup window but the user would like to save the raw spectrum automatically with the system-generated filename, the user needs to explicitly make sure that the *Automation->On* is checked.



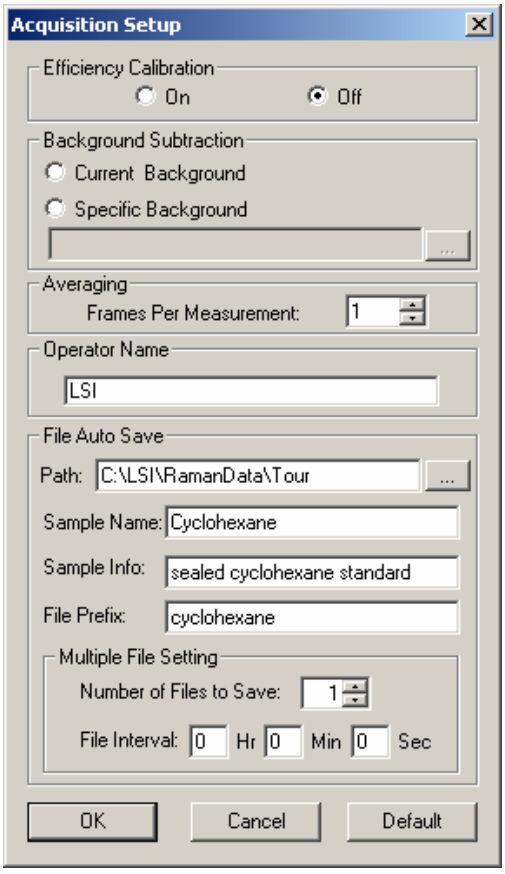
The Acquisition Menu

The Acquisition menu contains *Setup*, *Acquire*, *Continuous Acquire*, *Stop Acquire*, *Dark Current*, and *Background* submenu items. *Acquire*, *Continuous Acquire* and *Stop Acquire* are more easily

accessed from the Acquisition Window Toolbar buttons as discussed in the beginning of this chapter.

Setup

The Acquisition Setup window allows for setting up acquisition parameters before data acquisition is initiated.



Efficiency Calibration

The efficiency calibration (i.e., efficiency correction obtained through intensity calibration) can be chosen to be *On* or *Off*. This gives users the option to view the spectrum in the Acquisition Window with or without efficiency correction. The default setting is *Off*. Note that if the user has chosen *Background Removal* from the *System->Automation->Setup*, the efficiency correction is

then automatically applied and the process and efficiency-corrected spectrum is displayed in the Analysis Window.

Background Subtraction

The user can choose *Current Background* or *Specific Background*. To select a background subtraction method, click on the *Current Background* or *Specific Background* radio button. Click on the radio button the second time to de-select this background subtraction method.

If *Current Background* is chosen, the user is then asked to acquire a background spectrum by choosing *Acquisition->Background* immediately prior to acquiring the sample spectrum. This current background spectrum will then be subtracted from the acquired sample spectrum. Note that the background data buffer is always updated with the most recent background spectrum acquired through *Acquisition->Background*.

If *Specific Background* is chosen, the user can then browse to choose a background spectrum that has been previously acquired and saved into a file. To acquire a specific background spectrum and save it into a file, the user takes the same steps that would be used to acquire and save the spectrum of a real sample.

A background spectrum is a spectrum from a sample that produces (mainly) the fluorescence or reflected light background of an actual sample of interest. For example, the spectrum of water in a glass vial can be used as a background spectrum for dilute aqueous samples in the same type of glass vials. Appropriate background subtraction increases spectrum quality, and it is recommended that the background spectrum is taken with the same acquisition/processing parameters as the spectrum of the actual sample from which this background spectrum is to be subtracted.

Averaging

Averaging is controlled through *Frames Per Measurement* in which the user can set the number of frames to be averaged for each acquisition. Frame averaging is used to increase the signal-to-noise ratio of a sample with a low-Raman signal spectrum. Usually, an averaging of 3 to 5 frames would be enough. For very low-Raman signal spectrum, an averaging of up to 10 or 15 frames may be needed to provide good signal-to-ratio.

Operator Name

In the *Operator Name* field, the user can type in the name of the operator for this data acquisition.

File Auto Save

The *File Auto Save* group box allows the user to set up the file path/name for the spectrum data files to be saved.

We recommend that data are saved in the folder C:\LSI\RamanData, and create subfolders for each individual users. Note that the program can auto-generate a subdirectory on your computer hard disk specified on the *Path* field. Thus, if the user types in “C:\LSI\RamanData\User1” in the *Path* field, the program will create a subfolder “User1” within C:\LSI\RamanData, and the user does not need to actually go to C:\LSI\RamanData folder and create the “User1” folder.

Note that the program can only create one layer of new subdirectory. For example, it cannot create “C:\LSI\RamanData\User2\Chemicals” if the \User2 subfolder is not created yet.

The information that the user inputs in the *Sample Name*, *Sample Info*, and *File Prefix* fields, as well as that in the *Frames Per Measurement*, *Operator Name*, *Path* fields will all be included in the file header and in the report header.

Note that the spectrum data file name is generated by attaching a system date and time string (hour, minute, second) to the file name prefix that the user inputted in the *File Prefix* field. The program also attaches a numerical number "-1" at the end of the file name (also see *Multiple File Settings* below). If automation is activated through *System->Automation*, then the processed spectrum files are simultaneously saved with the same file name as the raw spectrum files, but with additional letters added: "BKSUB" for a background-subtracted spectrum, "B" for a background removed spectrum, "S" for a smoothed spectrum, and "N" for a normalized spectrum. For example, "cyclohexane0121124043-1.raw" is the filename for a raw spectrum file acquired in January 21, at 43 seconds past 12:40 pm, with the file prefix "cyclohexane". "cyclohexane0121124043B-1.pro" is the filename for the background-removed spectrum file corresponding to the above raw spectrum file.

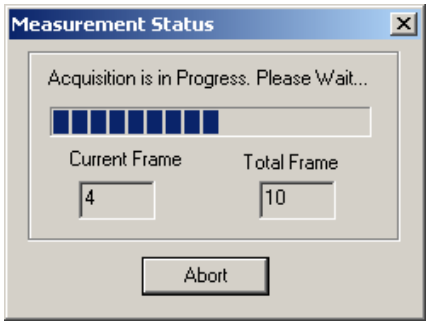
The *Multiple File Settings* group box offers the functionality of continuously acquiring and saving multiple spectra. The user can specify the number of files to be saved in *Number of Files to Save* field, and the interval between two consecutive acquisitions in *File Interval* fields. In this mode, the program attach a numerical number "-1", "-2", "-3", …, for the multiple files as a file index.

Acquire

When the user clicks on the *Acquire* submenu, or clicks on the 

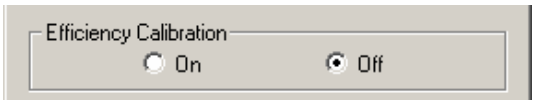
button on the Acquisition Window Toolbar, the system starts the data acquisition. The acquired spectrum will appear in the Acquisition Window, while the processed spectrum (if chosen through System Automation Setup) will be displayed in the Analysis Window.

Note that if frame averaging (see above subsection "Setup") is used, i.e., the number of frames per measurement is more than 1, a Measurement Status window will appear to inform the user the progress of the data acquisition.



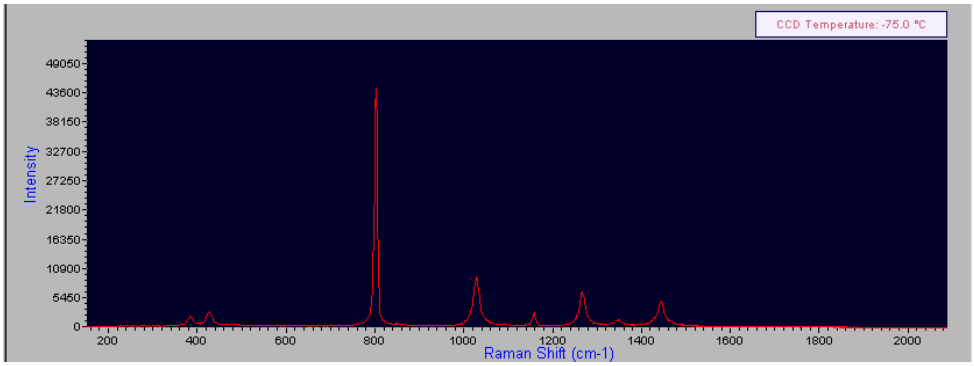
Acquiring with Efficiency Calibration On/Off

In the Acquisition Setup window, if the *Efficiency Calibration* is turned *Off*,

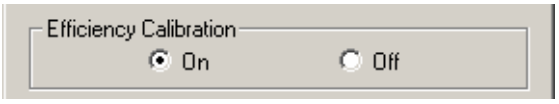


the spectrum acquired is a "truly" raw spectrum without efficiency correction, i.e., the intensity values are the raw counts from CCD camera readout without being corrected with the system

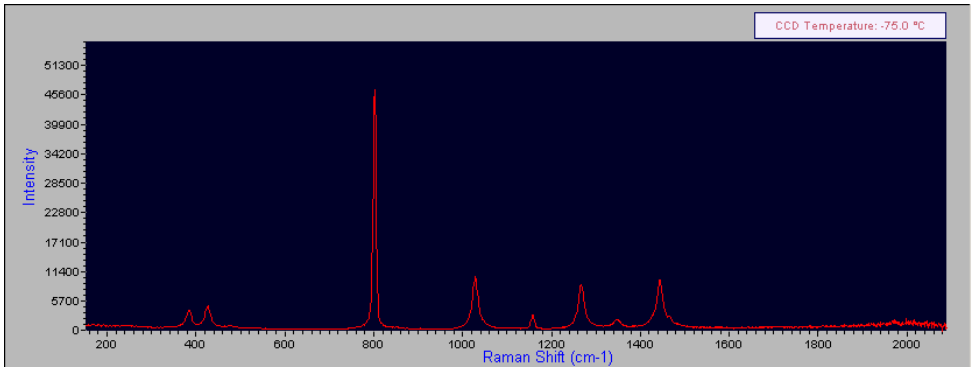
response (or, system efficiency). The following figure shows the cyclohexane spectrum in the Acquisition Window with efficiency calibration turned *Off*.



If the *Efficiency Calibration* is turned *On*,



the acquired spectrum is then efficiency corrected, i.e., the intensity values are corrected with the system response. The following figure shows the cyclohexane spectrum in the Acquisition Window with efficiency calibration turned *On*.



Note that the efficiency-calibrated spectrum appears noisier towards the higher wavenumber end of the spectrum (as well as in the beginning of the spectrum, but to a less degree). This is due to the fact that the system efficiency is “bell-shaped” (see section “Calibration”->”Intensity Calibration”), which means that calibrated spectrum is applied with increasingly larger multiplication factors towards the two ends, and thus the constant noise level in the raw spectrum is magnified towards the two ends.

Continuous Acquire / Stop Acquire

When the user clicks on *Continuous Acquire* submenu, the system starts to acquire the spectrum continuously, using the same CCD integration time for each acquisition. To stop continuous acquisition, click on the *Stop Acquire* submenu or press the 

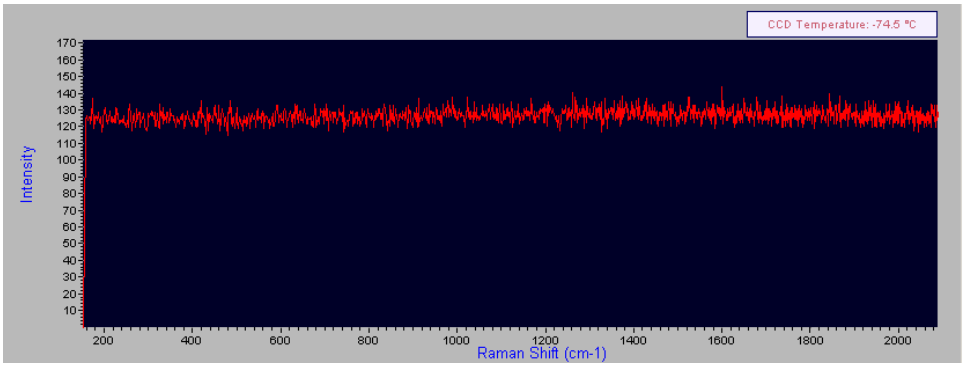
button on the Acquisition Window Toolbar. Note that in the continuous acquire mode, the spectra are not saved. These

spectra are generally used to obtain preliminary data while adjusting intensity or probe working distance.

Note: The acquisitions obtained through *Continuous Acquire* are single acquisitions; they are not multiple frames used for averaging (see Acquisition Setup).

Dark Current

When the user clicks on the *Dark Current* submenu, the system closes the laser shutter and performs a dark current measurement for the integration time and frames per measurement chosen. The shutter is turned on after the dark current is taken. Note that every time the CCD configuration (CCD temperature, integration time, acquisition speed, gain setting) and/or frame averaging are changed the system automatically re-takes the dark current prior to a data acquisition. The following figure shows a dark current spectrum. Here the first few pixels read lower dark current, and this is a characteristic of this specific CCD camera.



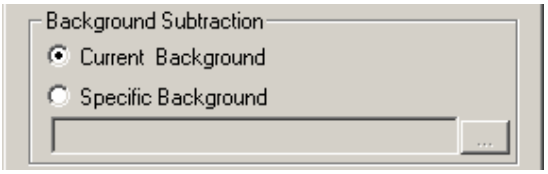
Background

The *Background* submenu allows the system to acquire a spectrum and save it into the background data buffer. This spectrum can also be saved into a file. If the *Background* submenu is clicked multiple times, the background data buffer is updated with the most recent background acquisition.

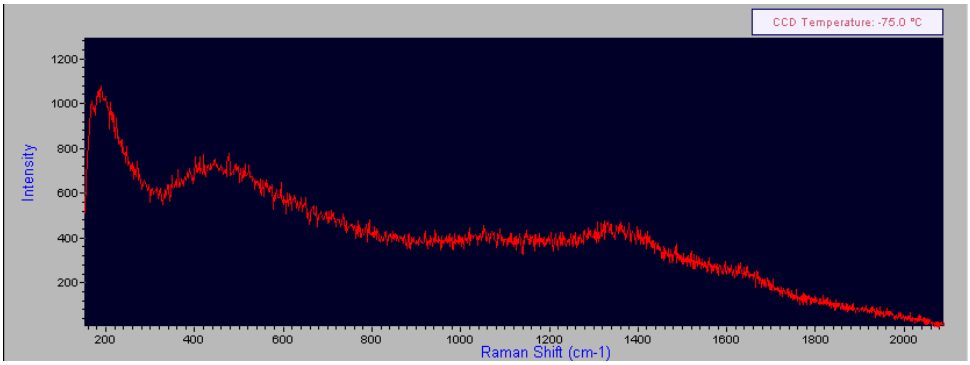
Acquiring a Spectrum with Current Background Subtraction

To acquire a sample spectrum and subtract a current background spectrum from the acquired sample spectrum, do the following:

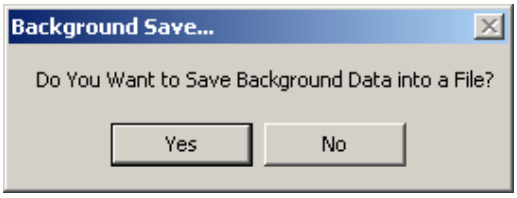
1. Put the “background sample” into the sample cell. In the *Background Subtraction* group box of the Acquisition Setup window, choose *Current Background*. Set other acquisition parameters such as laser power, CCD integration time, frame averaging.



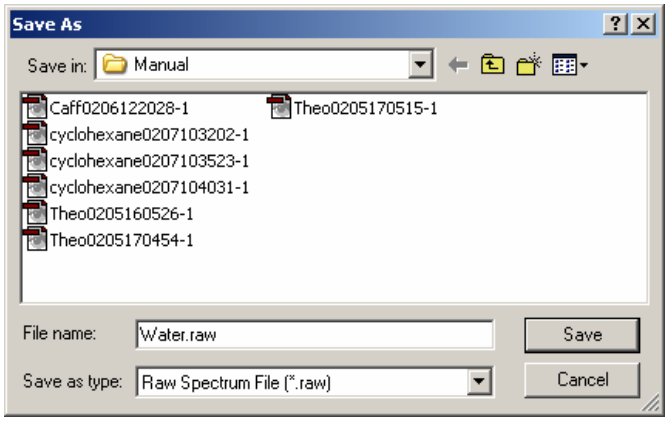
2. Click *Acquisition->Background*. The background spectrum is acquired and displayed in the Acquisition Window. The following figure shows an acquired background spectrum from water in a glass vial.



The user is asked whether to save this background into a file.

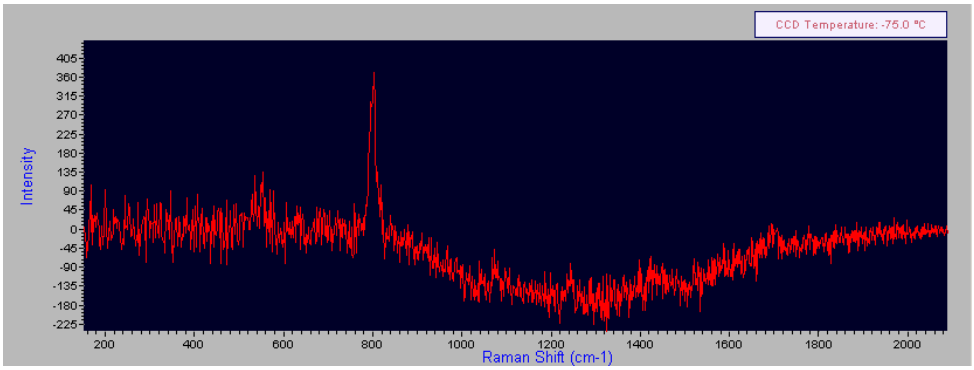


The user can click *No* to not to save the background, or click *Yes* to save it into a file for future use (for e.g., the saved background file can be used as *Specific Background* to be subtracted from other sample spectra. See next topic on “Acquire a Sample Spectrum with Specific Background Subtraction).

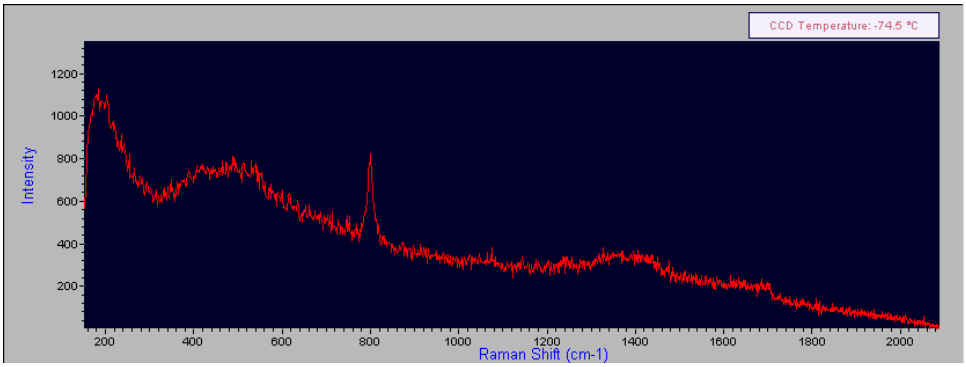


3. Put the sample of interest into the sample cell. In the Acquisition Setup window, set *Sample Name, Sample Info*, *File Prefix*. Be sure not to change other acquisition parameters (laser power, CCD Configuration, frame average) that you set up for acquiring the background.

4. Click *Acquisition->Acquire* or click on  on the Acquisition Window Toolbar. The background subtracted spectrum is displayed on the Acquisition Window.



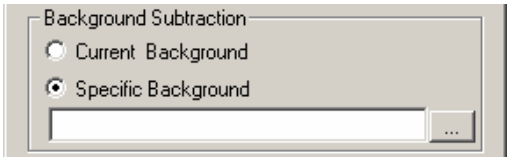
For the user's comparison, the original spectrum (10% aqueous acetone in glass vial) prior to background subtraction is shown in the following figure. In the above figure, the dip between 1000 – 1600 cm^{-1} could be due to an incomplete match between the measured sample and the background sample.



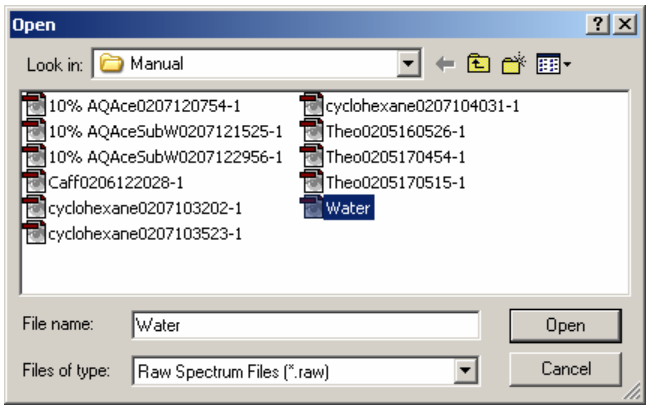
Acquiring a Spectrum with Specific Background Subtraction

To acquire a spectrum and subtract a specific background from it, take the following steps.

1. In the *Background Subtraction* group box of the Acquisition Setup window, click on *Specific Background* radio button. The textbox field beneath it becomes available.

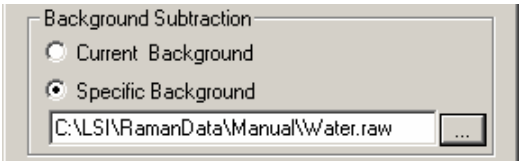


2. Press the browse button (**…**) to the right of the textbox field to locate a file that contains a background spectrum that the user intends to use. This background spectrum is acquired and saved into a file previously either through *Acquire->Background* or through an acquisition session just like with regular samples.



Note that the file that contains the background spectrum should be a raw spectrum file, i.e., of file extension .raw, since the program is to subtract this raw background spectrum from the acquired raw sample spectrum.

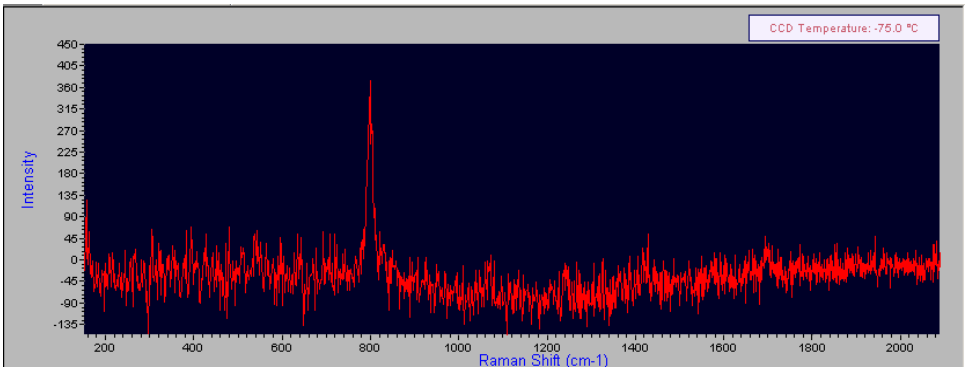
3. Click *Open*. The selected background spectrum file is shown in the text field.



4. Set acquisition parameters. It is strongly recommended that the user set the acquisition parameters (laser power, CCD configuration, frame average) as close as possible to those used when the background spectrum was taken. Set *Sample Name, Sample Info*, *File Prefix*.

5. Put the sample to be measured in the sample holder, click *Acquisition->Acquire* or press the 

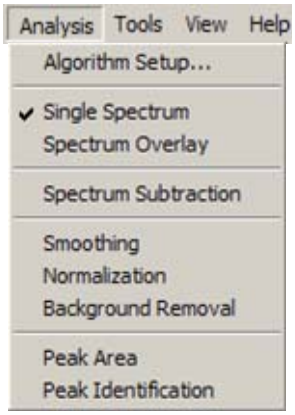
button on the Acquisition Window Toolbar. The acquired spectrum is background-subtracted and it is displayed in the Acquisition Window.



Note that if a spectrum is acquired with background subtraction from either current background or specific background, the spectrum is saved with both the original raw intensity data and the background intensity data, thus the raw spectrum and the background spectrum can be reconstructed from the saved data if desired.

The Analysis Menu

The Analysis menu includes the basic RamanSoft data processing and data analysis functions.



Some of these functions can alternatively be accessed through the Analysis Window Toolbar.

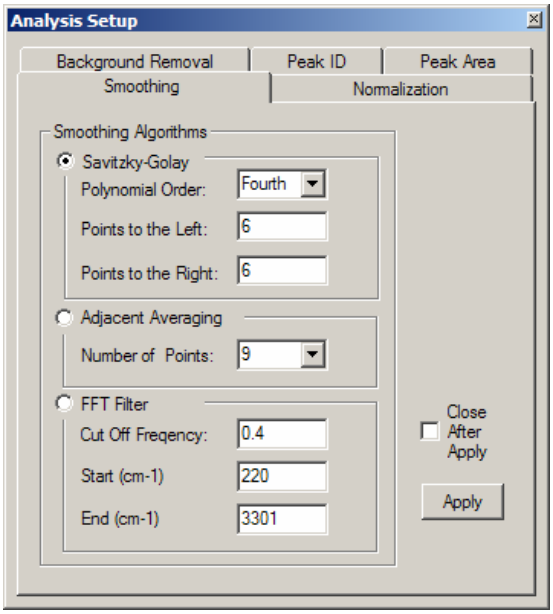
"**A**" stands for Algorithm Setup, "**S**" stands for Smoothing, "**N**" stands for Normalization, "**B**" stands for Background Removal, "**PI**" stands for Peak Identification, "**PA**" for Peak Area, and "**C**" clears the Analysis Window.

Algorithm Setup

The *Algorithm Setup* menu allows the user to configure one of the several available algorithms to apply for a particular data processing or data analysis function.

Smoothing

The smoothing algorithms include *Savitzky-Golay* (S-G) smoothing, *Adjacent Averaging* smoothing, and *FFT Filter* smoothing.



For applying S-G smoothing on Raman peaks, a *Polynomial Order* of 4, and both the number of *Points to the Left* and number of *Points to the Right* of 6 are recommended to maintain the original peak shape.

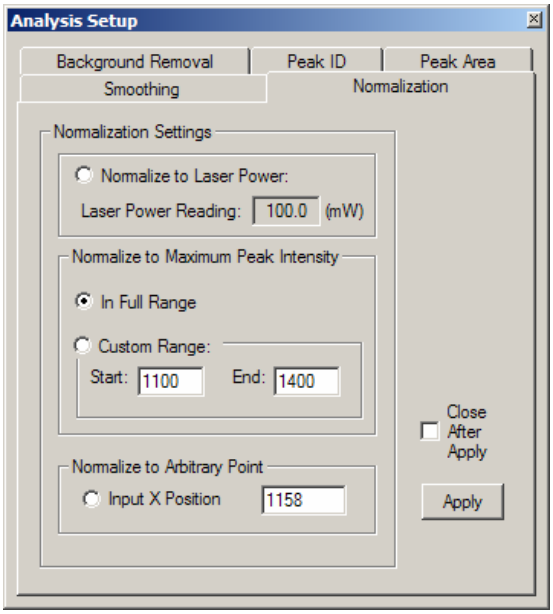
For *Adjacent Average* smoothing, a *Number of Points* of 3 or 5 is recommended so as not to distort the peak shape severely.

For *FFT Filter* smoothing, the range of *Cut Off Frequency* is from 0 to 1, where 0 provides the most filtering of the high frequency noises and 1 provides none. The *Start* and *End* gives the starting point and the ending point of the range of the spectrum where the FFT smoothing will be applied on.

The program sets the appropriate units (i.e., nm or cm^{-1}) for the *Start* and *End* points based on the x-axis display (set through *View->x axis*) in the analysis window.

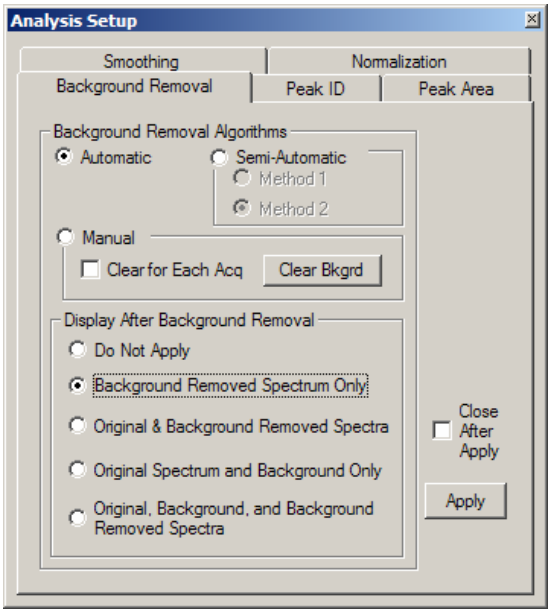
Normalization

There are four normalization options available. *Normalize to Laser Power* (as displayed in the *Laser Power Reading* field, this is the laser power at which the spectrum was taken); *Normalize to Maximum Peak Intensity* over the entire range of the spectrum (*In Full Range*); normalize to the maximum peak intensity in the customer chosen (x-axis) range (*Custom Range*); or normalize to the intensity value at any customer chosen spectrum point (*Normalize to Arbitrary Point*). Note that a spectrum can only be normalized to the laser power once.



Background Removal

There are three background removal methods: *Automatic*, *Semi-Automatic*, and *Manual* methods.



Automatic and Semi-automatic Background Removal

The *Automatic* method performs the default background removal automatically; the *Semi-Automatic* method gives the user two choices of algorithms to use: *Method 1* and *Method 2*. *Method 1* removes less background while retaining more small noisy peaks. *Method 2* performs more aggressive removal of background, but may remove some small peaks. In the *Automatic* method, *Method 1* is chosen if the intensity of the raw spectrum is less than 10,000, and *Method 2* is chosen if the intensity of the raw spectrum is greater than 10,000. Once an algorithm is chosen, click the *Apply* button.

Display Options

There are five display options after a spectrum is background-removed:

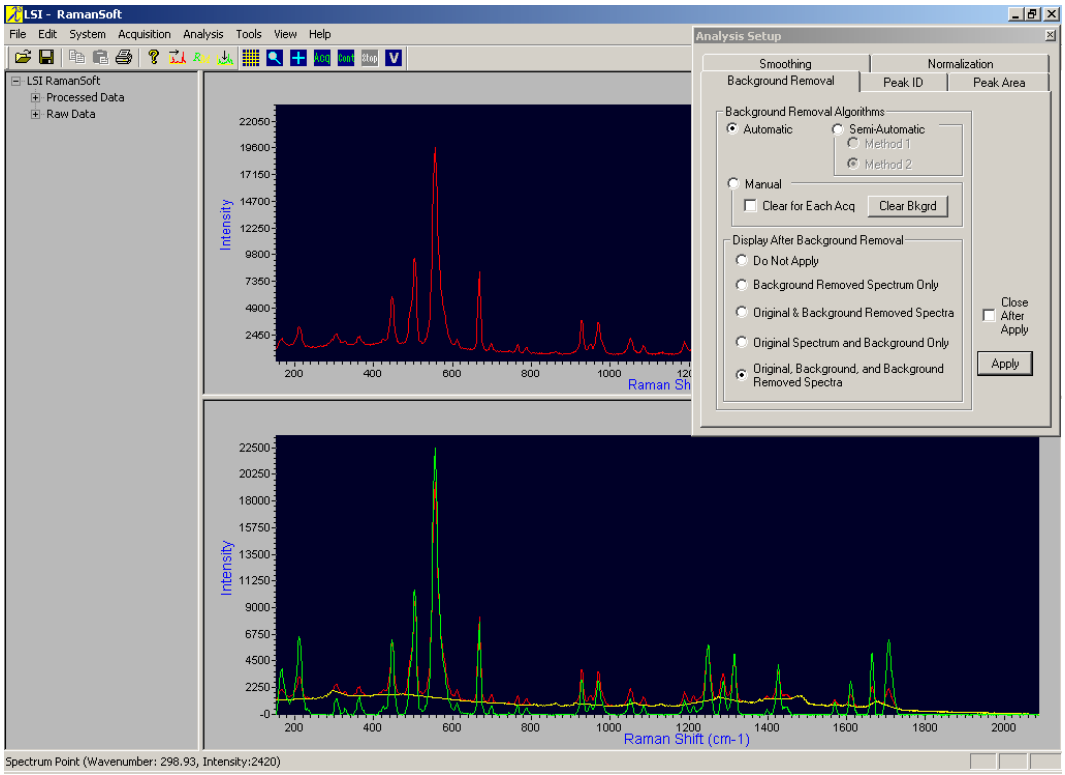
Option 1: *Do Not Apply*. Under this option only the original spectrum is displayed in the Analysis Window. This is equivalent to the fact that no background removal algorithm is applied. This display option can be used to reverse an earlier background removal action.

Option 2: *Background Removed Spectrum Only*. Only background-removed spectrum is displayed in the Analysis Window.

Option 3: *Original & Background-removed Spectra*. Both the original spectrum and the background-removed spectrum are displayed in the Analysis Window.

Option 4: *Original Spectrum and Background Only*. Both the original spectrum and the background generated either automatically under *Automatic* or *Semi-Automatic* mode or manually under *Manual* mode (see next topic "Manual Background Removal") are displayed in the Analysis Window.

Option 5: *Original, Background, and Background Removed Spectra*. Under this option, the original spectrum, the background either generated automatically under *Automatic* or *Semi-Automatic* mode or generated manually under the *Manual* mode (see subsection *Manual Background Removal*), and the background-removed spectrum are all displayed in the Analysis Window. The following figure shows such a display after an automatic background removal.



Manual Background Removal

The *Manual* background removal method gives users the option to perform background removal entirely manually.

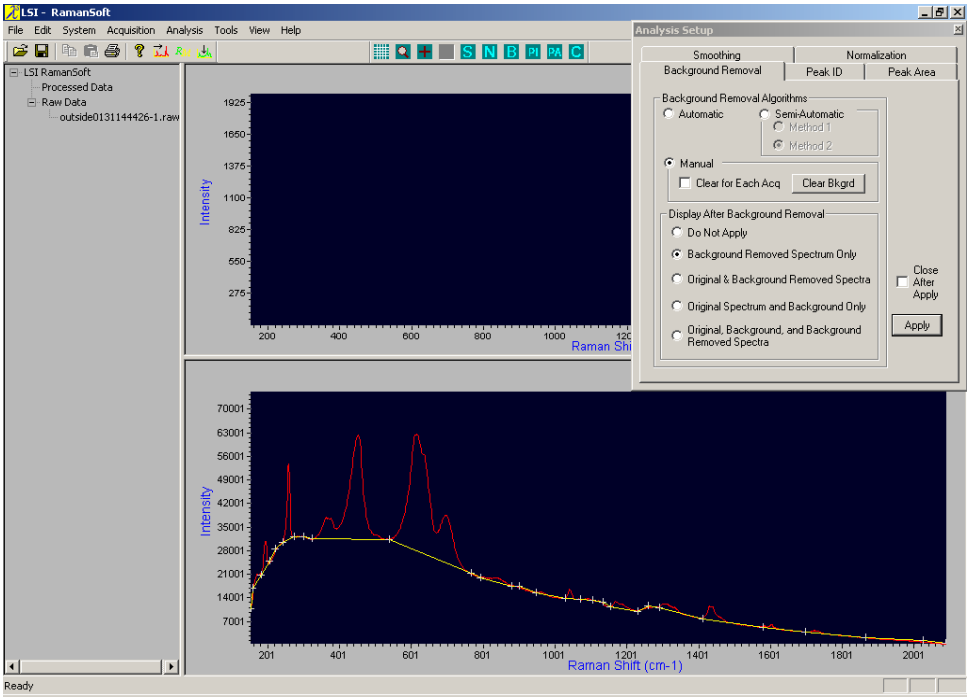
1. On the File View Window, click on the filename on the *Raw Data* list to display the spectrum to be manually background removed in the Analysis Window.

2. Click on *Analysis->Algorithm Setup->Background Removal* page tab; or click on  on the Analysis Window Toolbar and then click on the *Background Removal* page tab.

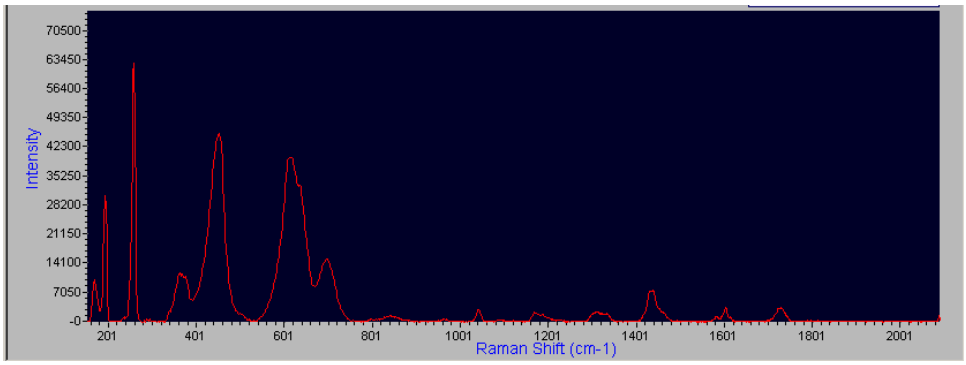
3. Check the *Manual* radio button, press *Clear Bkgrd* (a previous background will appear if there is one in the buffer), and click on the *Apply* button (this clears any previous background from the buffer).

4. Set the display option to Option 2: *Background Removed Spectrum Only* (or any other display options except the option *Do Not Apply*). Click on the *Apply* button.

5. Click left mouse at the start of the spectrum. The first point of the spectrum is automatically set as the first point of the background. Move the cross-line cursor to draw the background. When done, right-click the mouse. If the user right-clicked the mouse before the background was fully drawn till the end of the spectrum, the rest of the background will take the same Y-axis values as that of the last background point.



6. The background-removed (and also efficiency-corrected) spectrum is displayed in the Analysis window.



7. If unsatisfied, click on *Clear Bkgrd* button to clear the background, press *Apply,* and re-draw the background.

8. If the display option is set to Option 2, 4, or 5, the user can save the background-removed spectrum. Go to *File->Save As->Analysis Window*, and save it into a desired folder. It is recommended to add "_MB" to the end of the original file name to signify that the spectrum has been processed with the manual background removal method.

9. The user can also save the background itself into a file. To do this, choose the display option to be Option 3: *Original Spectrum and Background Only*. Go to *File->Save As->Analysis Window*, and save it into a desired folder.

10. The user can set to other display options to display the original spectrum, the background, and the background-removed spectrum.

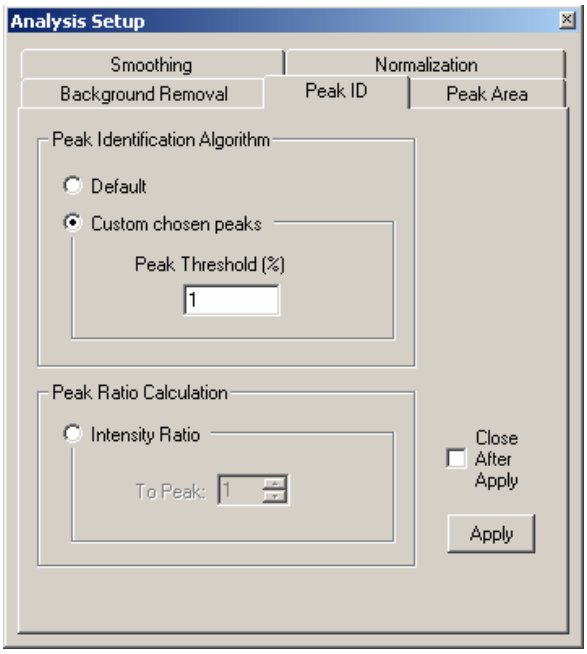
The *Clear for Each Acq* checkbox gives users the option to clear the manually-drawn background from the buffer. If this is not checked, and the *Manual* background removal method is chosen, then this background will be subtracted from all subsequently acquired spectrum.

Note: All background-removed spectra are automatically applied with efficiency correction. Thus, Background Removal should not be applied to a background-removed spectrum.

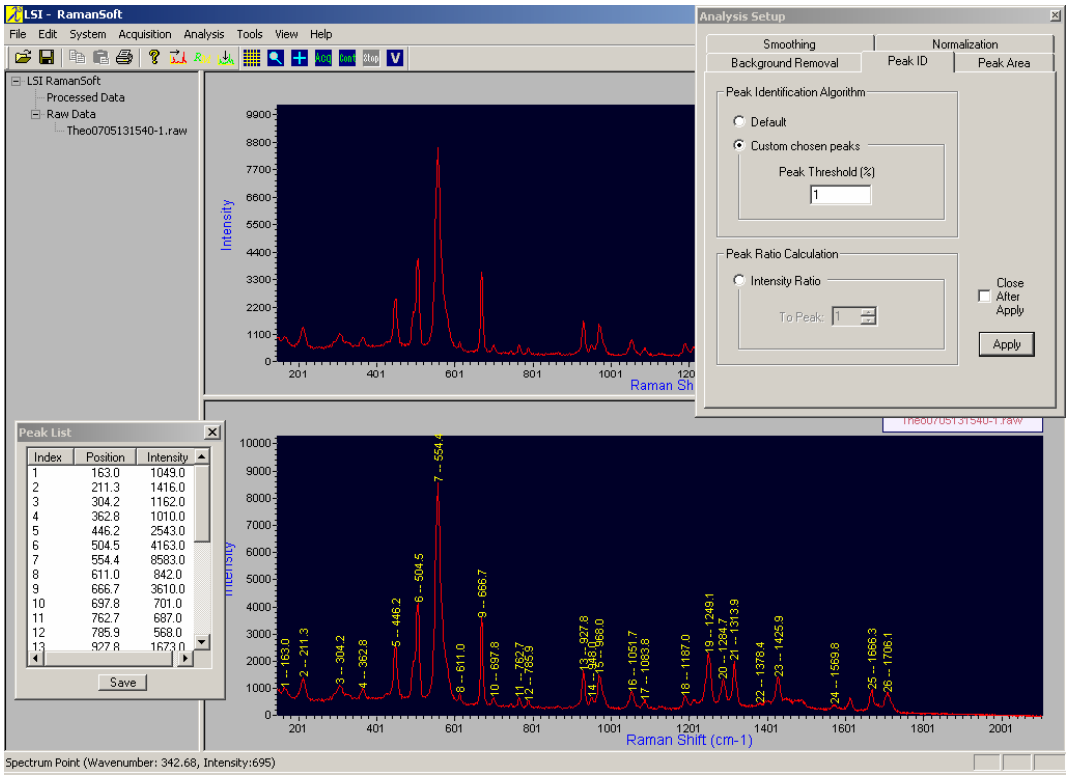
Note: If a data processing method (Smoothing, Normalization, Background Removal) is applied to a spectrum manually instead of through system automation while the spectrum was being acquired, the processed spectrum needs to be manually saved by choosing *File->Save As->Analysis Window* and entering a file name.

Peak Identification / Intensity Ratio

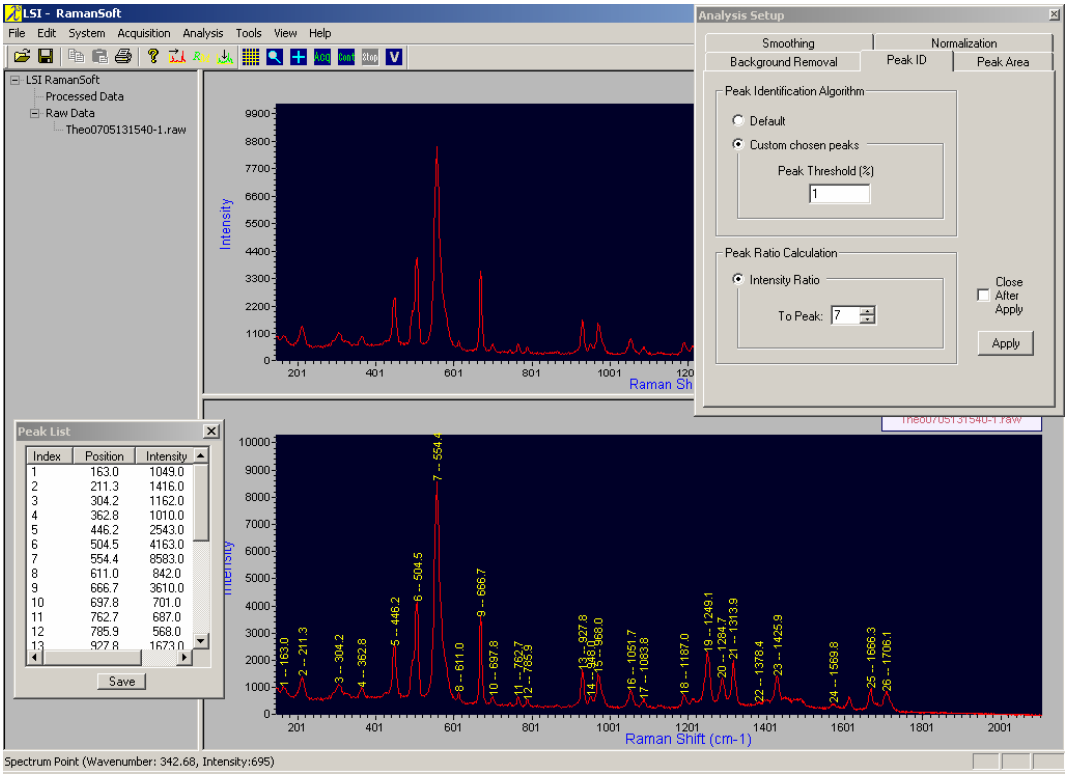
For peak labeling, the user can choose *Default* or *Custom chosen peaks* under the *Peak Identification Algorithm* group box. In the default mode, all peaks with an intensity greater than 5% of the maximum peak intensity will be identified. The user can choose other cut-off thresholds through the *Custom chosen peaks* option (such as 1%).



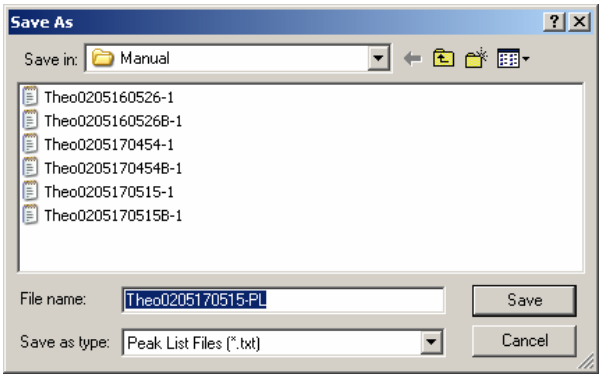
The following figure shows a peak-identified spectrum.



To obtain the peak ratio of the peaks, click on the *Intensity Ratio* radio button. The default ratio is to Peak 1; the user can change to any other peak through the arrow buttons or by typing in the peak index.



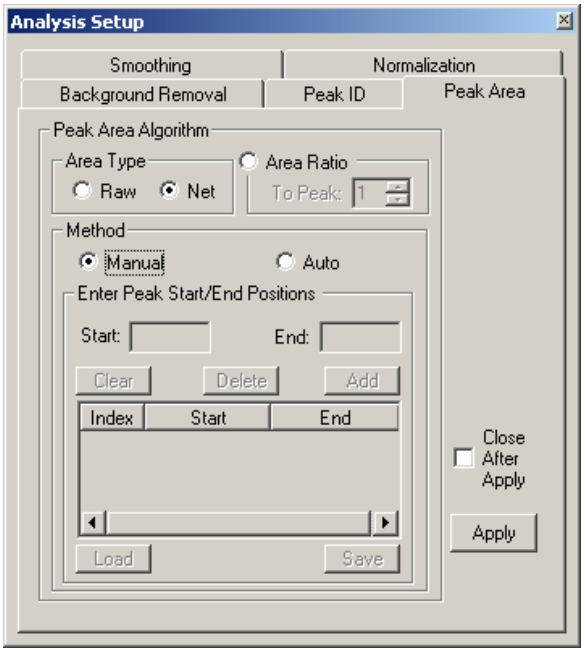
The contents (*Index*, *Position*, *Intensity*, and if intensity ratio is applied, the *Ratio*) of the *Peak List* table can be saved into a text file by clicking on the *Save* button. A filename is automatically generated, which is the name of the spectrum file with a "-PL" suffix.



Peak Area / Area Ratio

There are two ways of performing peak area calculation: the *Manual* method and the *Auto* method. The *Manual* method is the default method.

In the manual method, the user uses the cursor to click on the start and end points under a peak on the spectrum. In the auto method, the user inputs the start and end positions of peaks. These positions could have been saved into an Area List file, which could be loaded later for peak area calculation.



The user can choose to calculate *Raw* or *Net* area. The *Raw* area of a peak is the summation of all the intensity values of the spectrum points between the peak *Start* point and the peak *End* point on the x-axis. The *Net* area of a peak is the raw peak area minus the summation of all the intensity values of the baseline points, where a baseline is defined as the straight line that connects the peak *Start* point to the peak *End* point. The *Net* area type is set as the default area type.

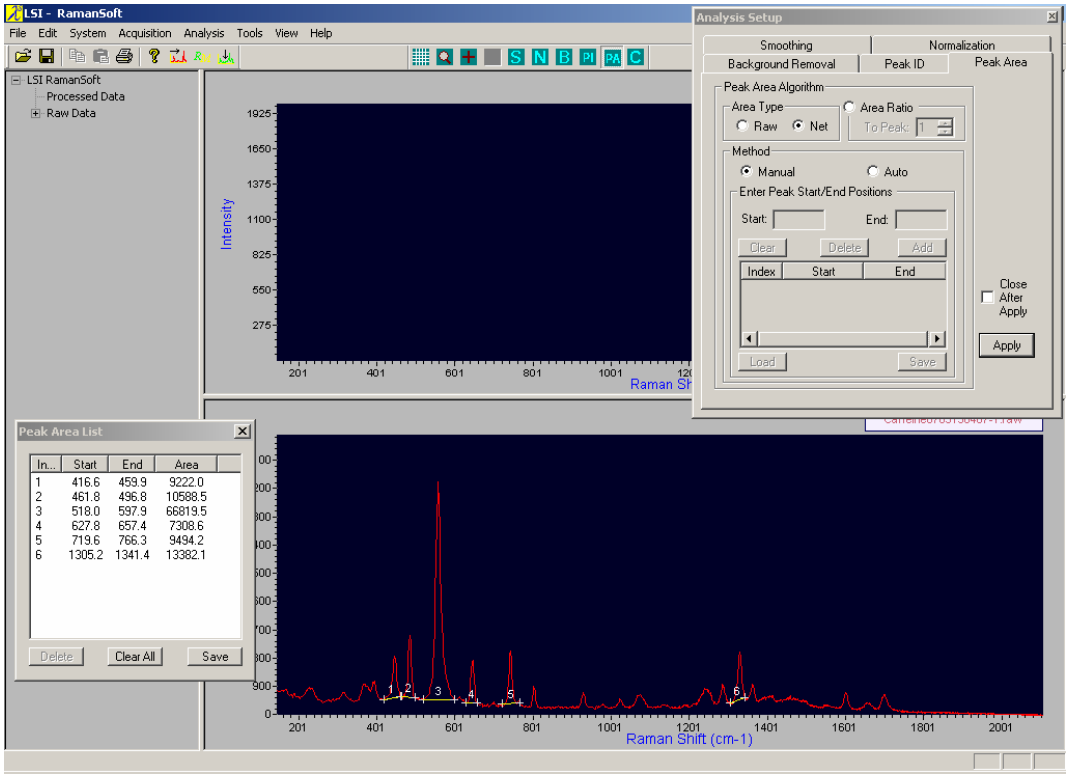
To obtain peak ratio by peak area, click on the *Area Ratio* radio button. The default ratio is to the first peak; the user can change to calculate the ratio to any other peak.

Manual Peak Area / Area Ratio Calculation

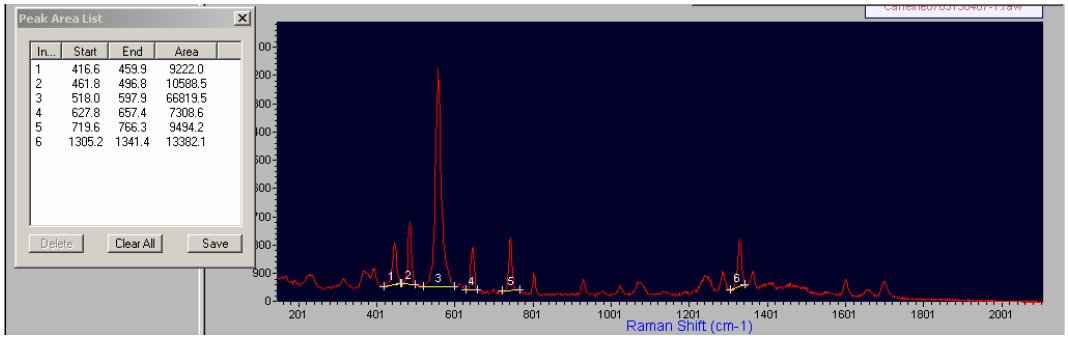
1. Click on the *Manua*l radio button, set the *Area Type* as *Raw* or *Net*, and click on the *Apply* button.

2. Define the peak start point and peak end point using left mouse click. When both the start point and the end point are set, a yellow line is drawn to connect the start point and end point, and an index number is marked under the peak. The calculated area of the chosen peak is displayed in the Peak Area List window.

3. Repeat step 2 to calculate the peak area of other peaks of interest. For each peak calculated, a row is added to the Peak Area List window, with each row consisting of an index number and the peak area value.

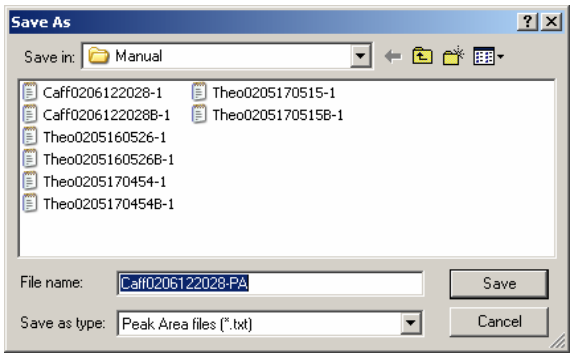


4. To remove a peak from the peak area calculation, the user can select the row that corresponds to that peak (as identified by the peak index) by clicking anywhere on the row in the Peak Area List window, and then press *Delete* button. This row is then removed from the Peak Area List window, and the corresponding peak is de-labeled in the Analysis Window. The remaining peaks are re-ordered in their index numbers. The below figure shows that peak 2 in the above figure is deleted.



5. To remove all peaks from the peak area calculation, press *Clear All* button.

6. All information displayed in the Peak Area List window (peak index, peak start position, peak end position, and peak area, and area ratio) can be saved into a text file. Click *Save* button. The program generates a default file name, which is the filename of the spectrum, plus a "-PA" suffix.

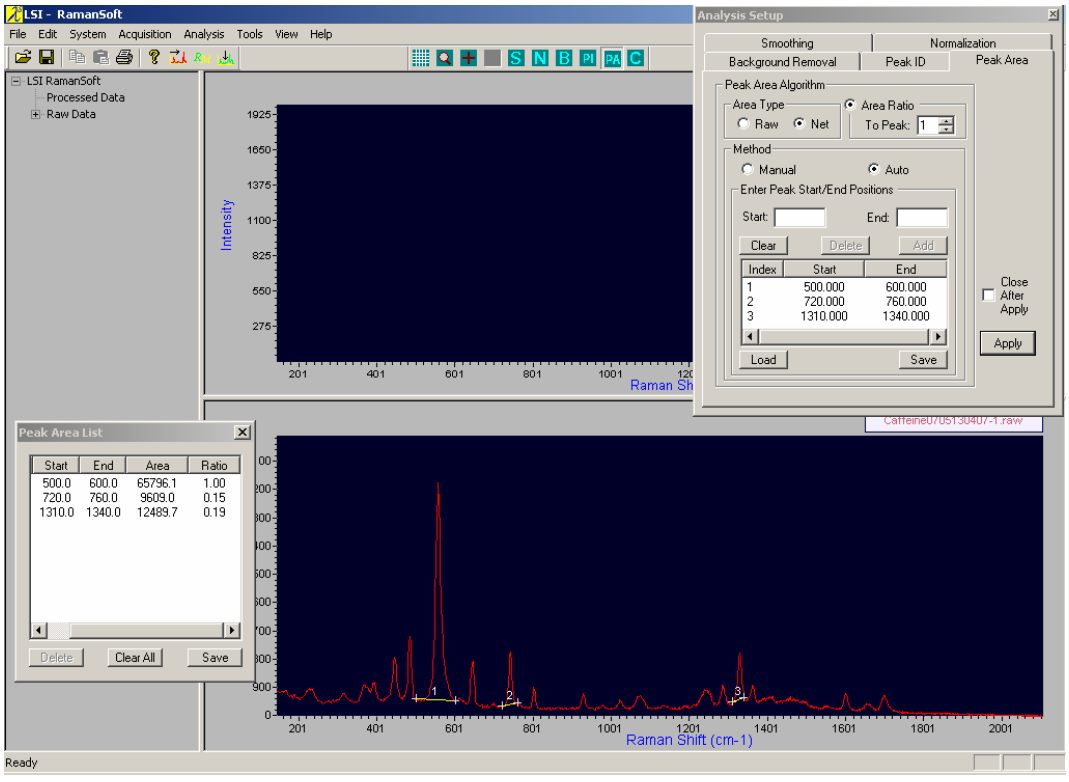


Auto Peak Area / Area Ratio Calculation - Input Peak Positions

1. Click on the *Auto* radio button, set the *Area Type* as *Raw* or *Net*, check *Area Ratio* radio button, and click the *Apply* button.

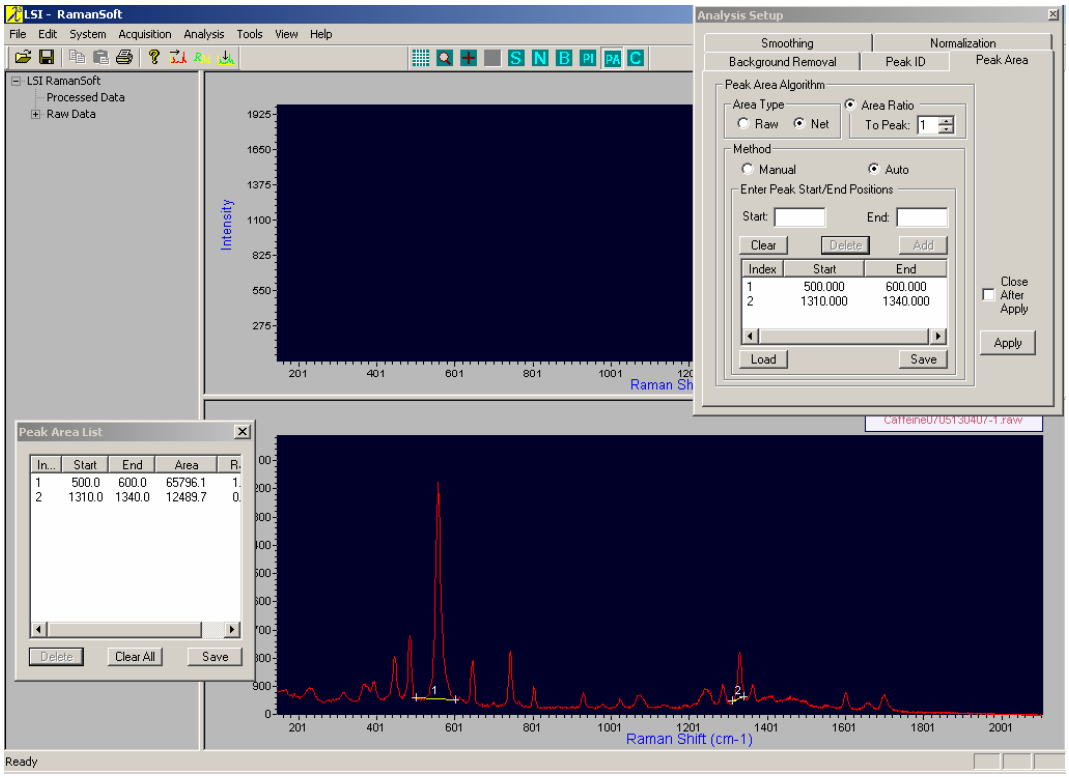
2. In the "*Enter Peak Start/End Positions*" group box, enter the peak start position and peak end position into the *Start* and *End* text box. Click *Add*. The peak start and end positions, along with an index number automatically assigned, is displayed in the lower text window. Repeat this procedure for other peaks of interest.

3. Click *Apply*. The peaks are labeled in the Analysis Window with the peak *Start* and peak *End* points connected with yellow lines. The calculated areas are displayed in the Peak Area List window.



4. To remove a peak from the peak area calculation, the user can select a row in the *Peak Start/End Positions* group box by clicking anywhere on the row in the text window to highlight this row, press *Delete* button, then click *Apply*. This row is then removed from the text window as well as the Peak Area List window, and the corresponding peak is de-labeled in the

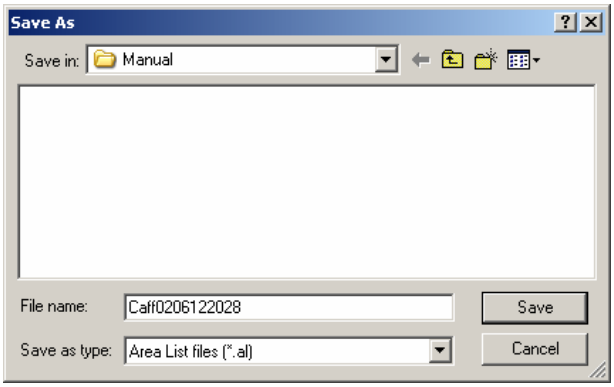
Analysis Window. Alternatively, the user can select the row that corresponds to that peak by clicking anywhere on the row in the Peak Area List window to highlight this row, and then press *Delete* button on the Peak List Window. The remaining peaks are re-ordered with new index numbers. The figure below shows that peak 2 in the above figure is deleted.



5. To remove all peaks from the peak area calculation, press *Clear* button in the Peak Area tab page or press *Clear All* button in the Peak Area List window.

6. To save the peak area calculation information, click *Save* button in the Peak Area List window to save the peak index, peak start position, peak end position, peak area, and area ratio into a text file. The program generates a default filename, which is the filename of the spectrum, plus a “-PA” suffix.

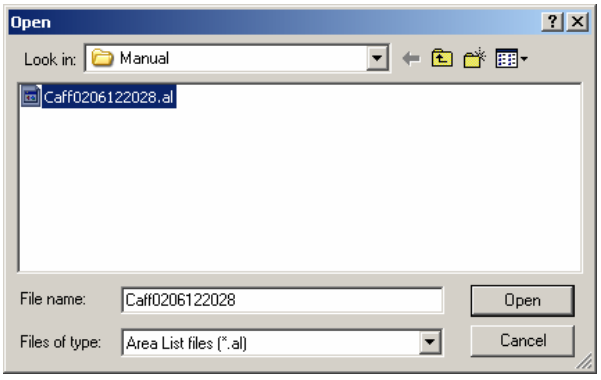
7. To save the peak start and peak end positions into a file (for future use), click *Save* in the Peak Area tab page. It can be saved with the same filename as that of the spectrum, but with a “*.al” extension (Area List file). This file can later be loaded into the text window of the Peak Area tab page for peak area calculation.



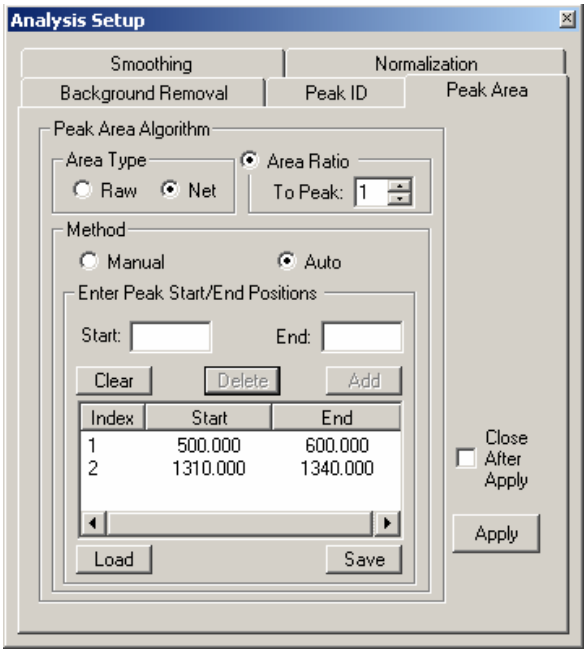
Auto Peak Area / Area Ratio Calculation – Load Area List File

1. From the Peak Area tab page, choose *Auto* method. Choose *Raw or Net* under the Area Type group box.

2. Click *Load*. Browse to locate the area list file that was previously saved (see above section *Auto Peak Area / Area Ratio Calculation – Input Peak Positions* on how to create an area list file), and click *Open* on the file Open window.



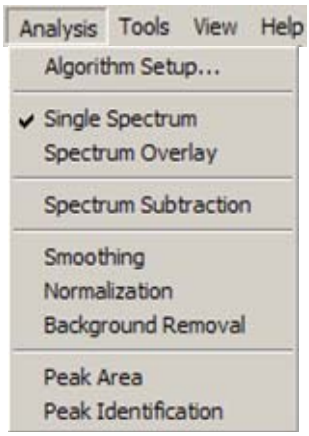
3. The peak start and end positions are listed in the text window of the Peak Area tab page.



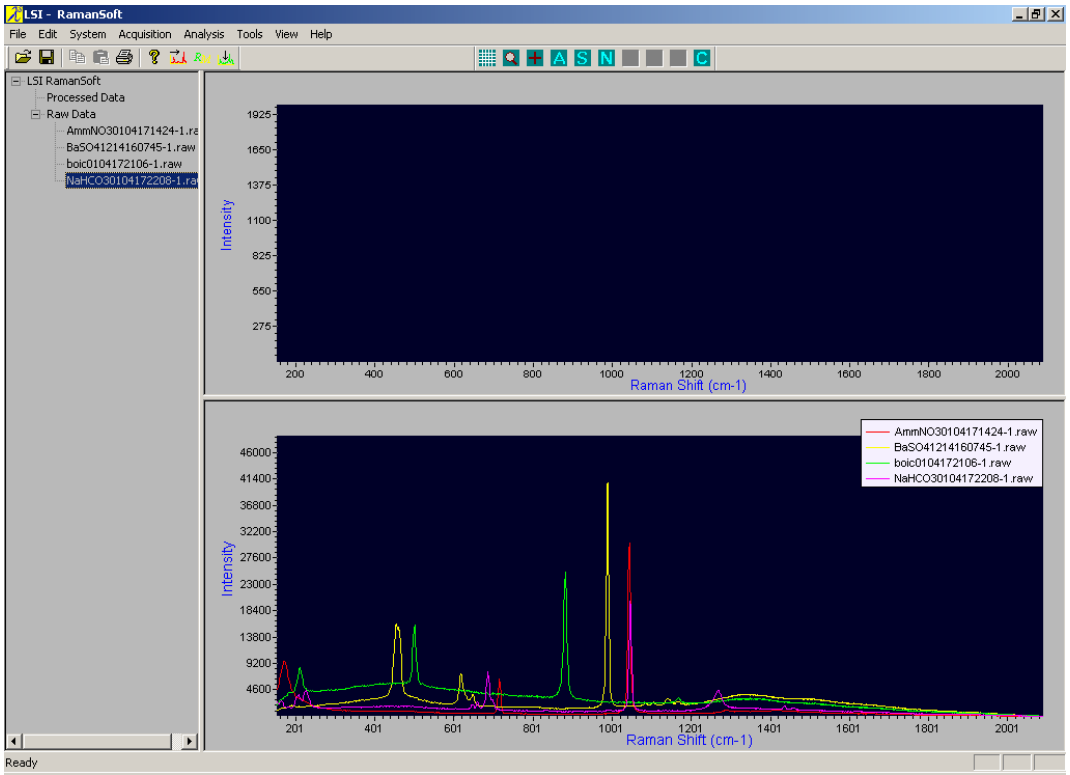
4. Follow Step 3 – 7 of the previous section *Auto Peak Area / Area Ratio Calculation - Input Peak Positions* to apply the peak area calculation.

Single Spectrum & Spectrum Overlay

In the *Single Spectrum* analysis mode (figure on the left below), only one spectrum can be displayed in the Analysis Window. All functions under the Analysis menu are available in the *Single Spectrum* mode. In the *Spectrum Overlay* mode (figure on the right below), up to six different spectra can be simultaneously displayed in the analysis window with each spectrum displayed in different colors. In this mode, only smoothing and normalization functions are available.



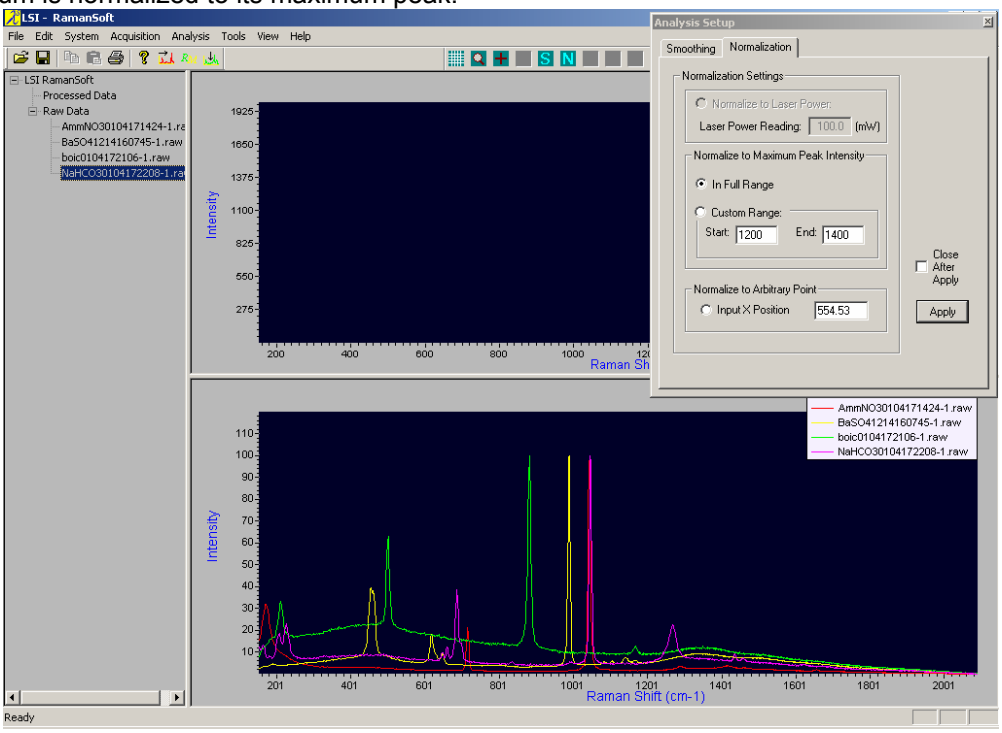
The following figure displays an overlay of four spectra. The legend and the filename of each spectrum is displayed in the upper right corner of the analysis window.



Note that on the Analysis Window Toolbar, only the smoothing and the normalization functions are available. To apply these functions, click on  to bring up the Analysis Setup window. Click on the *Smoothing* or the *Normalization* page tab to configure the smoothing or normalization

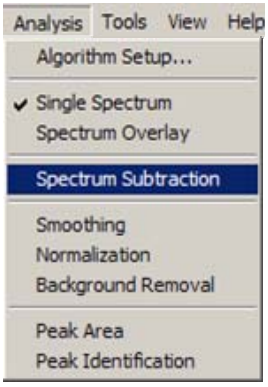
algorithm, and press the *Apply* button. The configured algorithm is then applied to all the spectra displayed in the analysis window.

The following figure shows the result of applying normalization "*In Full Range*", i.e., each spectrum is normalized to its maximum peak.

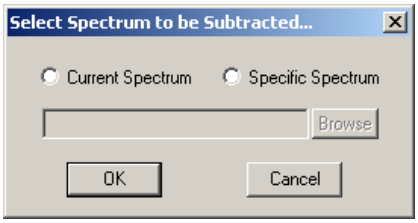


Spectrum Subtraction

This function allows the user to subtract one spectrum from another, a point-by-point subtraction of the intensity values of the second spectrum from that of the first one.

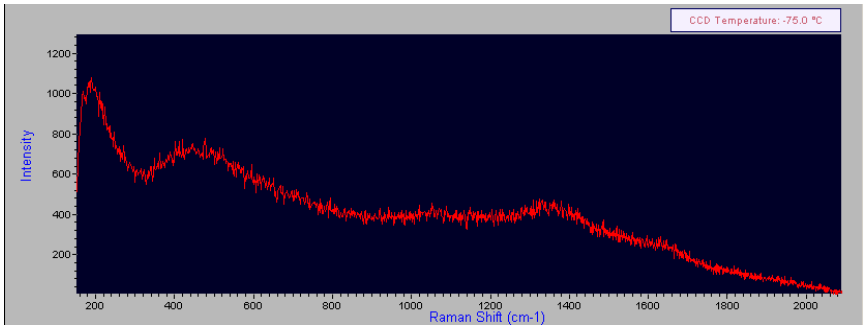


The spectrum to be subtracted from is the spectrum that is already displayed in the Analysis Window, and the spectrum to be subtracted can either be a currently acquired spectrum (*Current Spectrum*) being displayed in the Acquisition Window, or a spectrum retrieved from a file (*Specific Spectrum*).

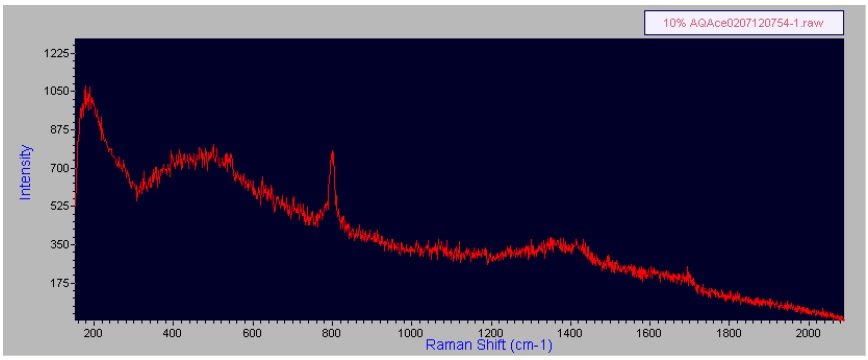


Subtracting a Current Spectrum

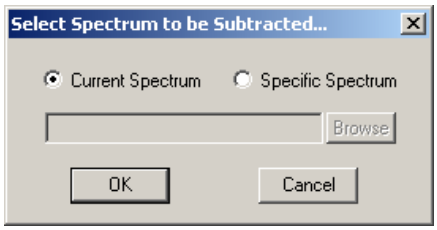
1. Acquire a spectrum that is to be subtracted. This spectrum is displayed in the Acquisition Window.



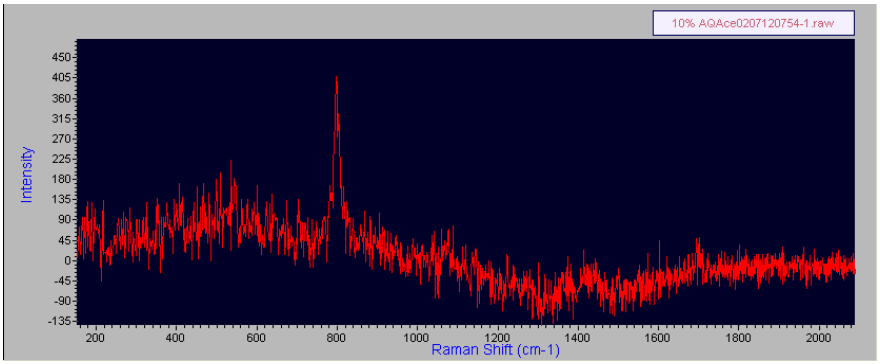
2. Retrieve the spectrum to be subtracted from and display it in the Analysis Window.



3. From the Select Spectrum to be Subtracted window, click the *Current Spectrum* radio button, and click *OK*.

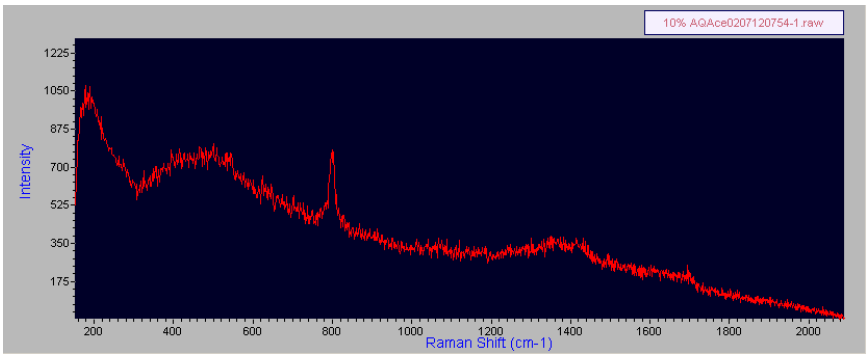


4. The subtracted spectrum is displayed in the Analysis Window.

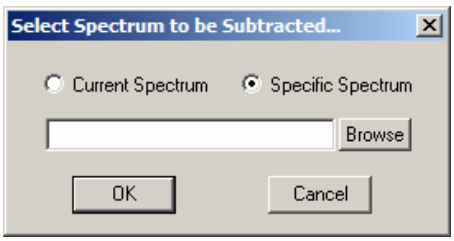


Subtracting a Specific Spectrum

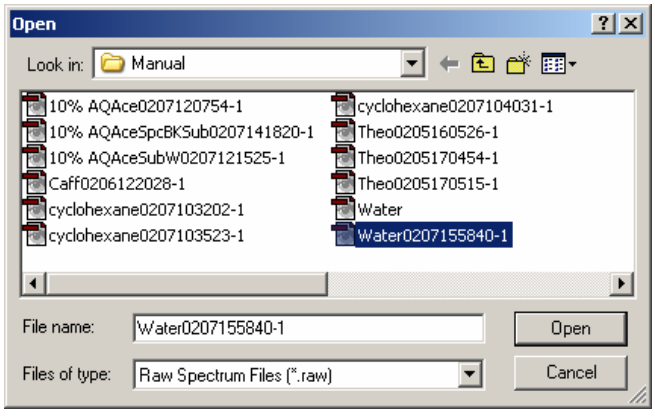
1. Retrieve the spectrum to be subtracted from and display it in the Analysis Window.



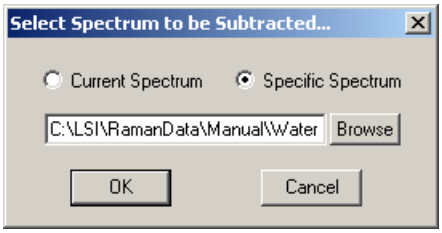
2. From the Select Spectrum to be Subtracted window, click the *Specific Spectrum* radio button, and click *OK*.



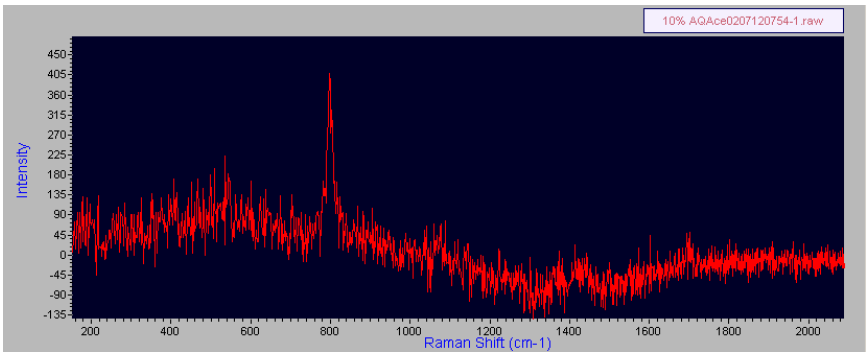
3. Click on the *Browse* button, a file open window is brought up to allow user to choose the specific spectrum file that was previously saved.



4. Once the user has selected the spectrum file, and clicked *Open*, the selected filename and path then appears in the “Select Spectrum to be Subtracted” window.



5. Click *OK*. The chosen “specific spectrum” is then subtracted from the original spectrum displayed in the Analysis Window.



Smoothing

The smoothing functions can be user-configured through the *Algorithm Setup -> Smoothing* tab page. Click on *Analysis -> Smoothing* to apply the configured smoothing algorithm to the spectrum displayed in the Analysis Window. Alternatively, the user can click on  on the Analysis Window Toolbar to apply the smoothing function.

Normalization

The normalization functions can be user-configured through the *Algorithm Setup -> Normalization* tab page. Click *Analysis -> Normalization* to apply the configured normalization algorithm to the spectrum displayed in the Analysis Window. Alternatively, the user can click on  on the Analysis Window Toolbar to apply the normalization function.

Background Removal

The background removal functions can be user-configured through *Algorithm Setup -> Background Removal* tab page. Click on *Analysis -> Background Removal* to apply the configured background removal algorithm on the spectrum displayed in the Analysis Window.

Alternatively, the user can click on  on the Analysis Window Toolbar to apply the background removal function.

Peak Identification

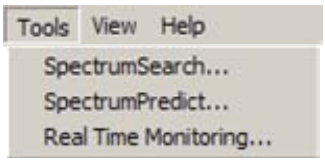
The peak identification functions can be user-configured through *Algorithm Setup -> Peak ID* tab page. Click on *Analysis -> Peak Identification* to apply the configured peak labeling threshold on the spectrum displayed in the Analysis Window. Alternatively, the user can click on  on the Analysis Window toolbar to apply the peak identification function.

Peak Area

The peak area functions can be user-configured through *Algorithm Setup -> Peak Area* tab page. Click on *Analysis -> Peak Area* to perform peak area calculation on the spectrum displayed in the Analysis Window. Alternatively, the user can click on  on the Analysis Window toolbar to apply the peak area function.

The Tools Menu

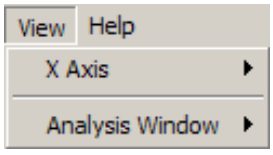
The three function modules, SpectrumSearch, SpectrumPredict, and Real Time Monitoring, are accessible through the *Tools* menu.



These modules can alternatively be accessed through the System Toolbar ( for SpectrumSearch,  for SpectrumPredict,  for Real Time Monitoring). Please refer to Chapters 6, 7, and 8 for the detailed functionalities of these modules, repectively.

The View Menu

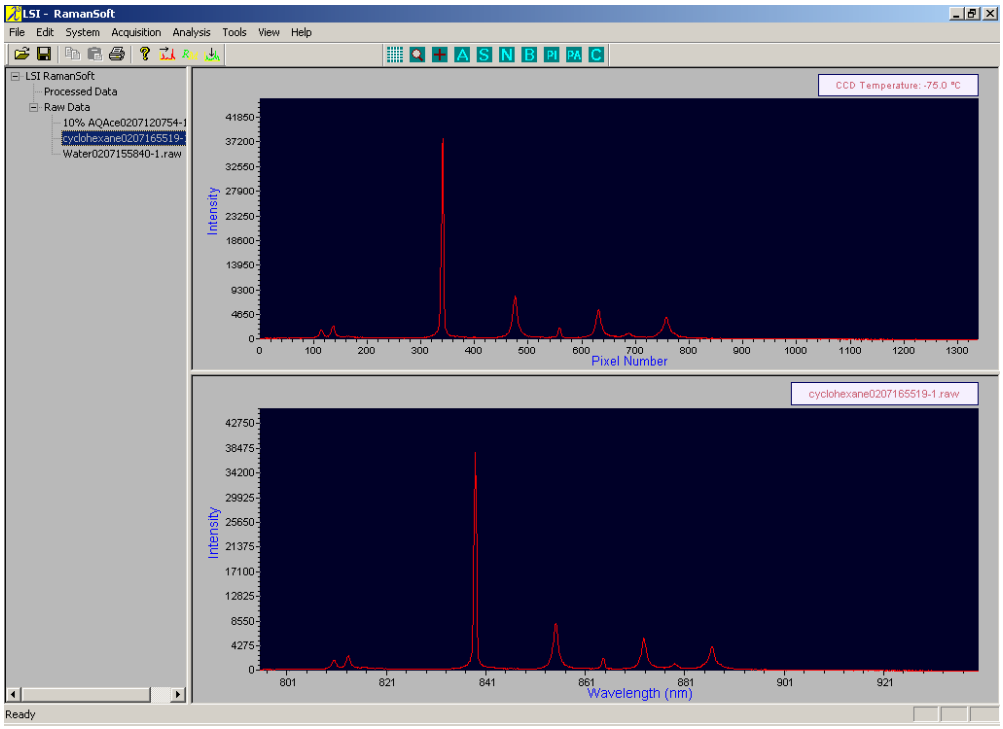
The *View* menu allows for configuration of various information display options.



X Axis

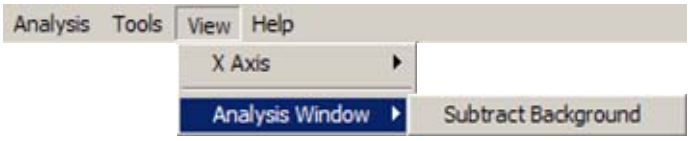
This option allows for displaying a spectrum in the Acquisition Window and/or a spectrum in the Analysis Window in different x-axis options; the spectrum can be displayed in Pixel Number, in Wavelength (nm), or in Wavenumber (cm^{-1}).

The following figure shows the cyclohexane spectrum displayed pixel number in the Acquisition Window, and in wavelength in the Analysis Window.



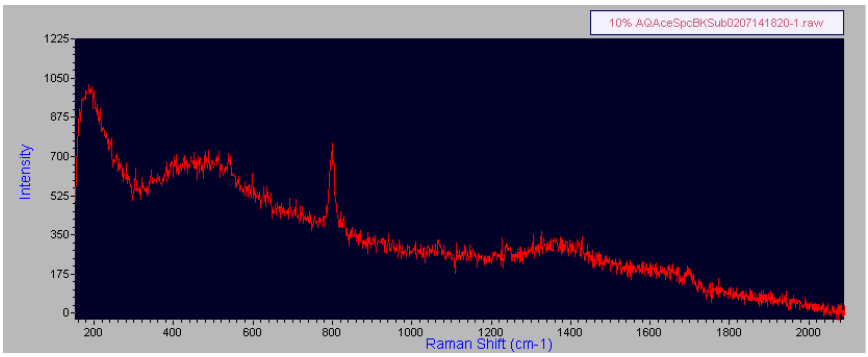
Analysis Window

This option allows for displaying the spectrum in the Analysis Window after subtracting the background data saved in the file (if background subtraction was used when the spectrum was acquired – see subsection “Background” under section “Acquisition” in this chapter).

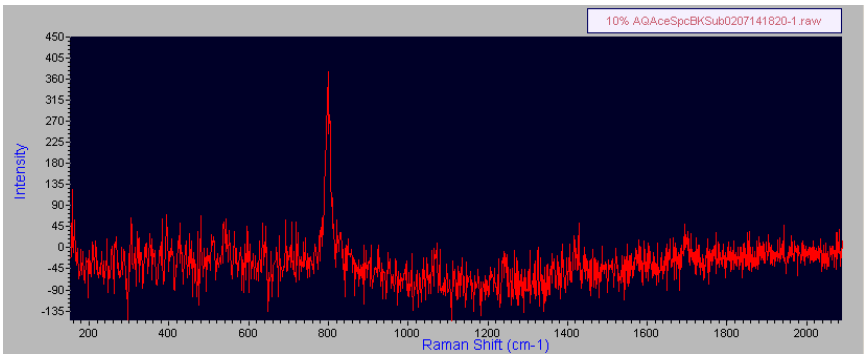


To use this feature, proceed with the following:

1. Open a file which has background data saved in it, and display it in the Analysis Window.



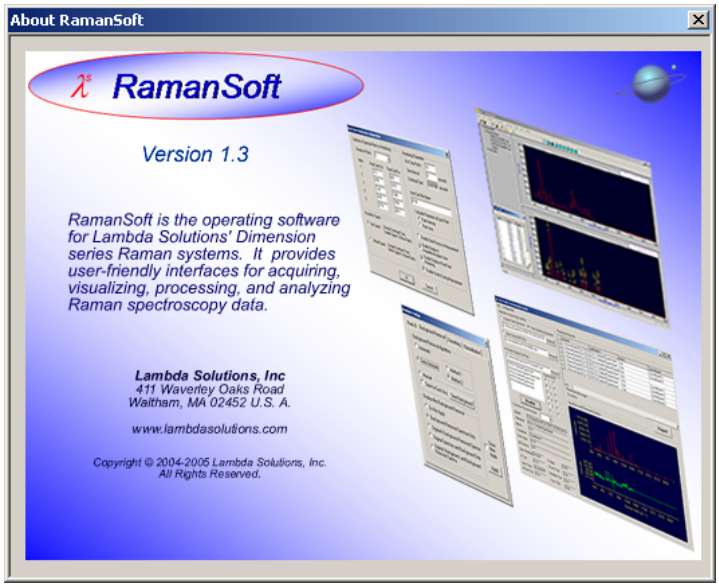
2. Click *View->Analysis Window->Subtract Background* (a check mark will appear to the left of the *Subtract Background* submenu item). The spectrum displayed in the Analysis Window is now background-subtracted.



3. To revert to view the original spectrum without subtracting the background, click *View -> Analysis Window->Subtract Background* again to uncheck the *Subtract Background* submenu item. The original spectrum without the background being subtracted will now be displayed in the Analysis Window.

The Help Menu

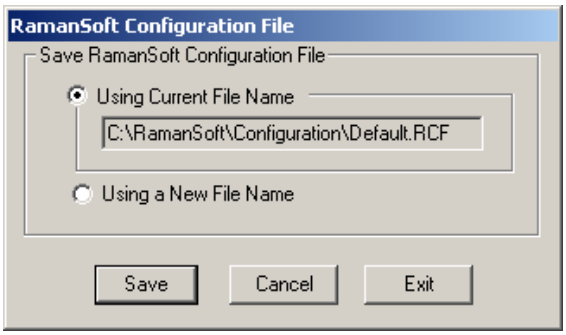
Currently the *Help* menu only contains one submenu item: *About RamanSoft*, which displays RamanSoft version/ copyright info. On-line help menu will be available for future versions. Registered users will be notified and updated.



Exiting RamanSoft

The user can exit RamanSoft by choosing *File->Exit*, or simply by closing the window.

When the user exits RamanSoft, the user is prompted to save the configurations used in the current RamanSoft session into the same configuration file or to save it in a new file. The default setting is to save it into the same configuration file.



Exiting RamanSoft Without Saving Configuration

Press *Exit* to exit RamanSoft without saving the configurations.

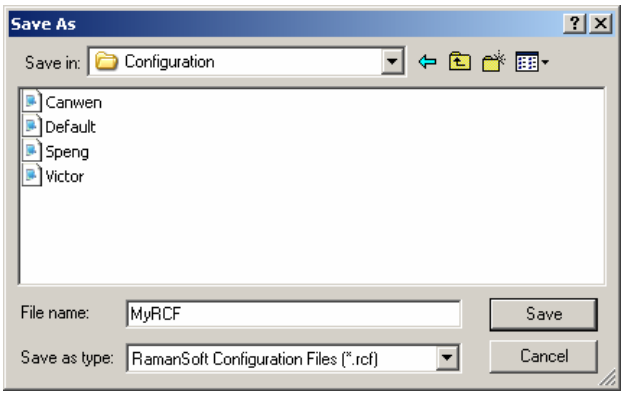
Saving Configuration Into Current File

Since this is the default setting, just click *Save* to save the configurations into the same file name as displayed in the *Using Current File Name* textbox. RamanSoft is then exited.

Saving Configuration Into a New File

1. Click the *Using a New File Name* radio button. Press *Save*.

2. The Save As window appears. Browse to C:\LSI\RamanSoft\Configuration (where the Default.rcf configuration file is located), and input the new configuration file name.



3. Click *Save*. A new configuration file is then created (in the above figure, a new configuration file "MyRCF.rcf" is created). The program is exited.

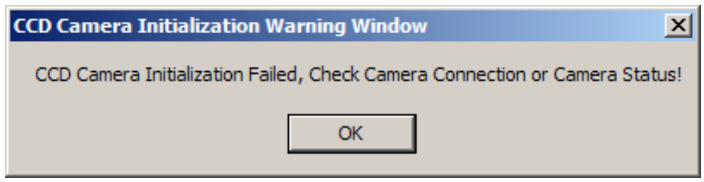
If a user chooses the Default.rcf when starting RamanSoft, it is recommended that the user save the configurations into a new file so that the user can conveniently retrieve his/her own preferred settings for data acquisition, processing, and analysis in a future RamanSoft session.

RamanSoft Trouble Shooting

This section describes the trouble shooting protocols for the RamanSoft basic functionalities.

CCD Camera Communication Failure

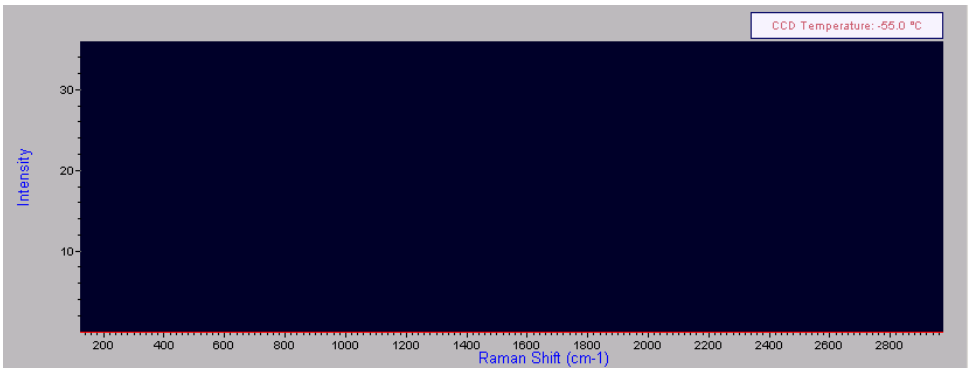
If the CCD Camera Initialization Warning Window appears when RamanSoft is first started:



The USB 2.0 cable between the Dimension-P Raman unit and the computer may not be properly connected. Connect the USB 2.0 cable, make sure that the two ends of the USB cable is push-tight, and restart RamanSoft.

If the USB 2.0 cable is connected properly, and the above window still appears, there could be one RamanSoft application already started, in which case there should be two RamanSoft icons displayed on the Windows task bar. Close the second RamanSoft application.

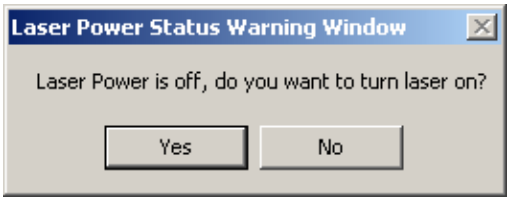
If the second RamanSoft application is used to acquire data despite the CCD camera communication failure warning, no data will be acquired (as the CCD can only communicate to one active RamanSoft application), and the Acquisition Window would display the following:



Note that if the USB 2.0 cable is disconnected during a RamanSoft session and the CCD Camera Initialization Warning Window appears, the user needs to exit the program and restart RamanSoft application to reinitiate the communication between the CCD camera and the computer.

Laser Power Status Warning

If the system has been idle (i.e., without acquisition action) for more than 30 minutes, the system turns the laser off automatically. A subsequent acquisition action would invoke the Laser Status Warning Window.



Click *Yes* to turn the laser on (the Laser Power Stabilizing Progress Window will appear), and then click 

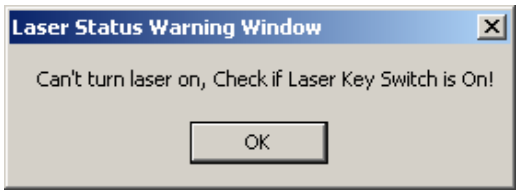
or 

to continue the acquisition action.

Click *No* to remain at the laser power off status. A spectrum taken without laser on would appear like white noise spectrum (see the figure in subsection “White Noise Spectrum Acquired”).

Laser Status Warning

If the Laser Status Warning Window shows up (most often when the user first start RamanSoft but forgot to turn the Laser Key to ON position),



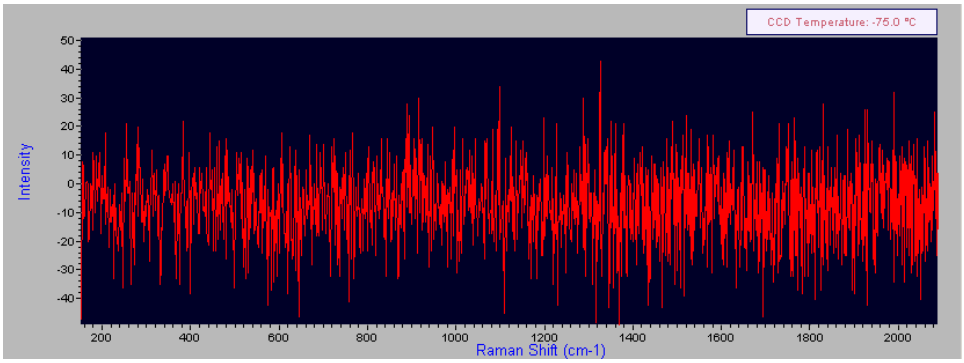
The warning indicates that the laser is not turned on yet, i.e., the Laser Power key is at OFF position (on the front panel of the Dimension-P Raman).

Click *OK* on the Laser Status Warning Window, turn the Laser Power Key to ON position on Dimension-P Raman.

Note: If the Laser Key Switch is at ON position and nonetheless the Laser Status Warning Window appears (clicking OK in the Laser Status Warning Window and subsequent acquisition action will keep bringing up the Laser Power Status Warning Window and the Laser Status Warning Window), there could be a loss of communication between the computer and the Dimension-P Raman, such as a disconnection of the USB 2.0 cable. In this case, exit RamanSoft, connect the USB 2.0 cable, and restart RamanSoft.

White Noise Spectrum Acquired

If the spectrum acquired appears to be a white noise spectrum in the Acquisition Window,

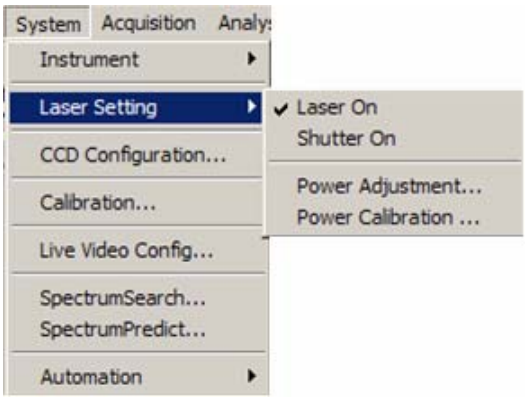


and the sample is placed properly in the internal sample cell or the External Sampling Module and that it is believed to have a Raman signal, the laser might be off (the program turns the laser off automatically if the system has been idle for more than 30 minutes). Go to *System->Laser Setting*, and make sure that *Laser On* is checked. If not, click on *System->Laser Setting->Laser On* to turn the laser on. See the following section “Turn Laser On From RamanSoft” for more details.

Turning Laser On From RamanSoft

Even if the Laser Power key switch is in the ON position, there may still be no laser power output. The laser is turned on automatically in the following two cases: a. When the Laser Power key switch is turned to ON position before RamanSoft is launched. b. When there is an acquisition (either through *Acquire* or *Continuous Acquire*). The system automatically turns the laser off if there has been no acquisition for the past 30 minutes.

To turn laser on from RamanSoft, go to *System->Laser Setting*, and make sure that *Laser On* is checked. If not, click on *System->Laser Setting->Laser On* again to turn the laser on.


System
Acquisition
Analy
Instrument
Laser Setting
CCD Configuration...
Calibration...
Live Video Config...
SpectrumSearch...
SpectrumPredict...
Automation
Laser On
Shutter On
Power Adjustment...
Power Calibration ...

6 The SpectrumSearch Module

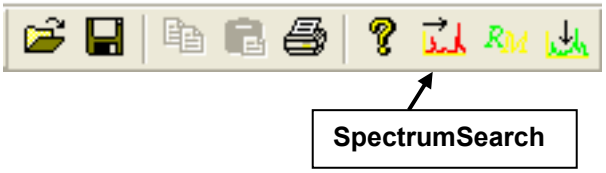
Overview

The SpectrumSearch module is an integration of RamanSoft with the Thermo Electron Corporation's Spectral ID® functionality for seamlessly performing sample spectrum acquisition and spectral library searching in one step in order to rapidly identify unknown samples. The integrated system allows the user to set up experimental parameters, acquire spectra to build a new library or add a spectrum to an existing library, and perform spectral identification in such a way so that the user only needs to work through the RamanSoft SpectrumSearch interface. Acquisition parameters such as CCD integration time and frame averaging are set up in the RamanSoft main menu (see Chapter 5, RamanSoft Basic Functionality).

Launching the SpectrumSearch Application

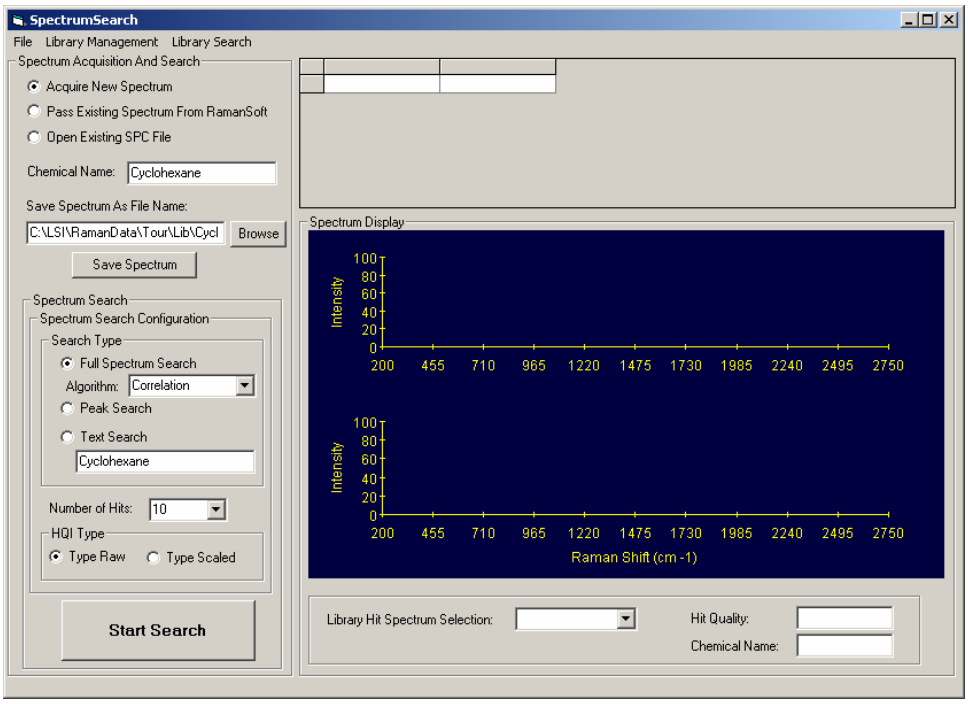
The SpectrumSearch module can be launched through the *Tools->SpectrumSearch* menu of the RamanSoft main menu.

It can also be started by clicking on the SpectrumSearch icon  on the Function Toolbar.



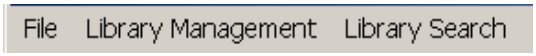
The SpectrumSearch User Interface

The main user interface for the SpectrumSearch module appears as follows:



The selections on the left panel of the interface may differ every time you start the SpectrumSearch application as it depends on the saved contents of the configuration file and thus reflects information from a prior session.

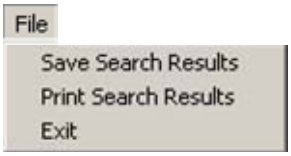
The main menu of the SpectrumSearch module consists of *File*, *Library Management*, and *Library Search*.



The function of each menu is described in the following sections, together with the Spectrum Acquisition and Search functions that can be configured through the SpectrumSearch interface.

The File Menu

The file menu contains *Save Search Results*, *Print Search Results*, and *Exit* menu items.



Save Search Results

This allows for saving the library search results as a text file. Note that the search results are already automatically saved (as a text file) each time a search session is commenced. The *Save*

Search Results menu gives the user an option to save the file using any other filename/location in addition to the automatically saved file.

Print Search Results

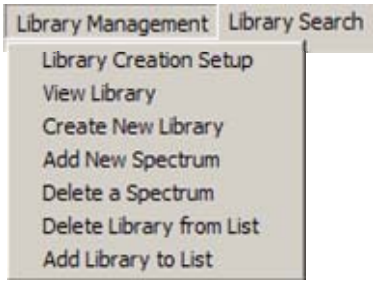
This allows for printing the search results saved in the text file to a printer.

Exit

This exits the SpectrumSearch module.

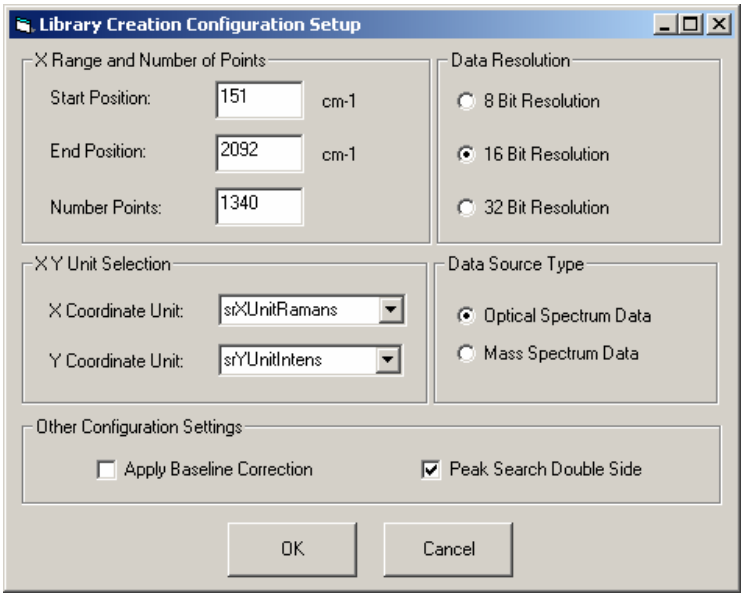
The Library Management Menu

The Library Management menu contains all functionalities for creating and maintaining a library.



Library Creation Setup

The Library Creation Configuration Setup window lets the user configure the parameters for creating a new library.



X Range and Number of Points

The *Start Position* and *End Position* specify the starting and ending wavenumbers for all the spectra in the library. The default setting uses the full spectral range. This is usually desired and it is also recommended when the library is first created. If the user wants to match a limited spectral region of the unknown sample spectrum to the spectra in the library, the user can choose to search a specified region of the library spectra through the *Select Search Spectrum Region* function in *Library Search->Spectrum Search Setup*.

Data Resolution

The Data Resolution specifies the resolution of the spectra stored in the library. 8 bit resolution means that the intensity values of a spectrum is rescaled from 0 to 255. Usually, *8 Bit Resolution* is sufficient but 16 and 32 bit resolution can be chosen. The higher the resolution, the more precision is retained in the spectral data, but the slower the search would be. In SpectrumSearch, 16 bit is set as default.

When an unknown spectrum is searched against the library, the unknown spectrum is scaled according to the same resolution as the library spectra.

XY Unit Selection

The XY Unit Selection allows for the selection of x-axis and y-axis units. “Raman Shift (cm^{-1})” is the default for the *X Coordinate Unit*, and “Intensity” is the default for the as *Y Coordinate Unit*.

Data Source Type

The default mode for Data Source Type is *Optical Spectrum Data*. For SpectrumSearch, do not select *Mass Spectrum Data*.

Other Configuration Settings

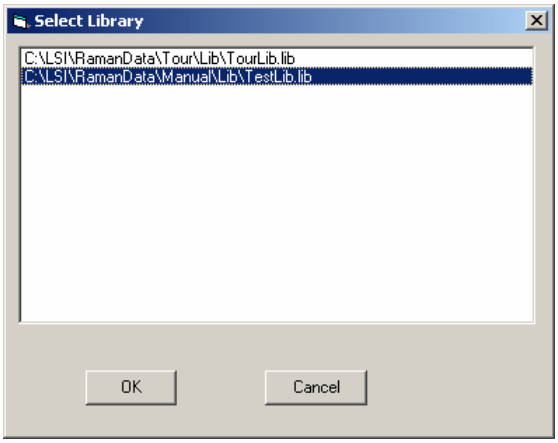
If *Apply Baseline Correction* radio button is checked, the GRAMS’ Auto Level Baseline Correction algorithm is applied to each spectrum when it is added into the library. This method fits a best straight baseline to the non-peak areas of data. Thus it can only remove a linear (slope) background, and it cannot remove arbitrary background shapes.

Please Remember ***not to*** check this option if RamanSoft Automatic Background Removal is enabled.

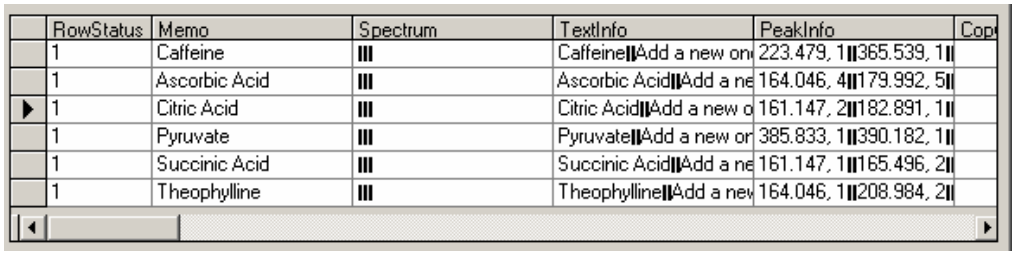
The user can choose to use *Peak Search Double Side* search algorithm for *Peak Search*. The default algorithm is Single Side Peak Search algorithm, in which the peaks from the unknown sample spectrum are compared to the peaks in a library spectrum with only the ascending side of the peak. For Double Side Peak Search, the peaks are compared with both the ascending side and the descending side. Double side peak search should give more accurate match between the unknown spectrum and the library spectra.

View Library

When *Library Management->View Library* is chosen, the Select Library window appears, which lets the user choose a library to be viewed. Note that there could be more than one library listed in Select Library window (if more than one library is added to the library list – see Section “Add Library to List”), choose the one that is to be viewed by clicking anywhere on that item to highlight it.



Click *OK*. All the entries in this library are then listed in the top right section of the SpectrumSearch interface.



	RowStatus	Memo	Spectrum	TextInfo	PeakInfo	Cop
	1	Caffeine	|||	Caffeine||Add a new on	223.479, 1||365.539, 1|	
	1	Ascorbic Acid	|||	Ascorbic Acid||Add a ne	164.046, 4||179.992, 5|	
▶	1	Citric Acid	|||	Citric Acid||Add a new o	161.147, 2||182.891, 1|	
	1	Pyruvate	|||	Pyruvate||Add a new or	385.833, 1||390.182, 1|	
	1	Succinic Acid	|||	Succinic Acid||Add a ne	161.147, 1||165.496, 2|	
	1	Theophylline	|||	Theophylline||Add a new	164.046, 1||208.984, 2|	

The above table contains the following information for each library entry:

RowStatus — The status of the entry. 1 indicates “OK” status, “4” indicates a “Deleted” status, i.e., the spectrum has been deleted from the library.

Memo — The text information that the user inputs in the *Chemical Name* field of the SpectrumSearch interface when the spectrum is first acquired to be added to the library.

Spectrum — The actual spectrum of the library entry. Displayed as “|||” (i.e., not displayed) in SpectrumSearch.

TextInfo — The text information for the library entry, consisting of the contents in the memo field, plus information such as “Add a New One”, which is not displayed when the TextInfo field of a library entry is clicked.

PeakInfo — The peak positions and relative intensities (on a scale of 0 to 9) of all the peaks of a library spectrum extracted by SpectrumSearch (Spectral ID®).

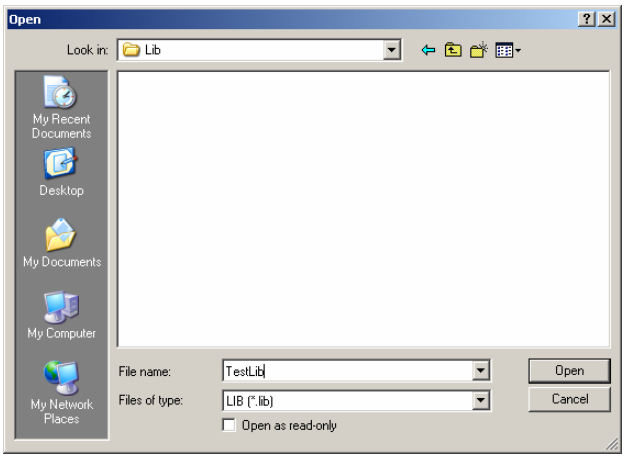
CopyRight — The copyright info if the library or library spectrum is imported from a copyrightable source.

MolFile — The molecular structure file related to the library entry.

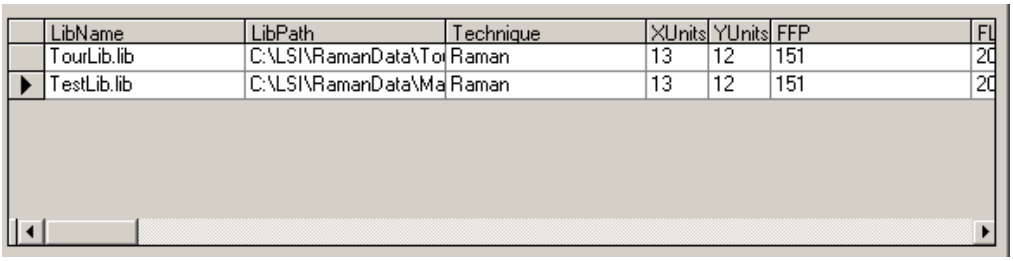
DBPointer — The related database source.

Create New Library

From *Library Management*, choose *Create New Library*. Browse to a folder into which the library is to be placed and enter a name for the library.



Click *Open*. The new library is created but empty. The name of the library is displayed in the top right section of the SpectrumSearch user interface.



LibName	LibPath	Technique	XUnits	YUnits	FFP	FL
TourLib.lib	C:\LSI\RamanData\To	Raman	13	12	151	20
TestLib.lib	C:\LSI\RamanData\Ma	Raman	13	12	151	20

Note that the program lists all the libraries that are currently on the library list. When a new library is created, this library is automatically added to the library list (see “Add to Library List” in this section). If another library (“TourLib.lib” in the above figure) is previously added to the library list and not deleted from the library list (see “Delete Library From List” in this section), then this library still remains on the library list.

LibName	The file name of the library
LibPath	The file path of the library
Technique	The technique (“Raman”) employed in the hardware system.
XUnits	X coordinate unit. “13” corresponds to “Raman Shift (cm^{-1})”
YUnits	Y coordinate unit. “12” corresponds to “Intensity”
FFP	The x coordinate of the first point in the library spectra
FLP	The x coordinate of the last point in the library spectra
Comment	The comments for the library. Currently fixed as “Test Library”.
Selected, LibRows	These fields are left empty.

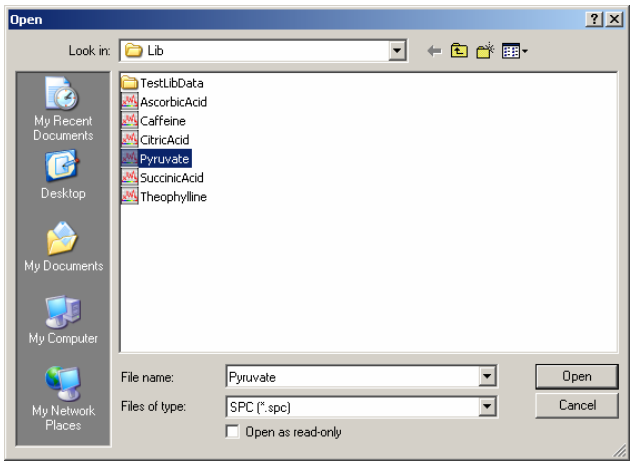
Some of the above columns (*XUnits, YUnits, FFP, FLP*) display the settings configured in the Library Creation Configuration Setup window (see Section “Library Creation Setup”)..

Note that when the library is created, a set of files (*.idx, *.ipl, *.isl, *.itl, .lib) is created along with the library file (.lib) and these files are located in the same directory as the lib file. These files are required for library management and library searching. However, the user only needs to use the lib file.

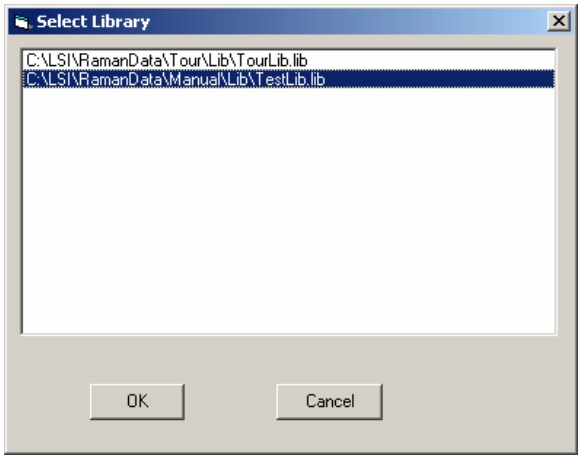
Add New Spectrum

This allows for adding a new spectrum to a library that is already created. The library could be empty (like when it is first created) or could already have other spectra in it.

From *Library Management*, choose *Add New Spectrum*. Browse to the folder where the spectrum (in .SPC format) is located, select the spectrum and click *Open*.

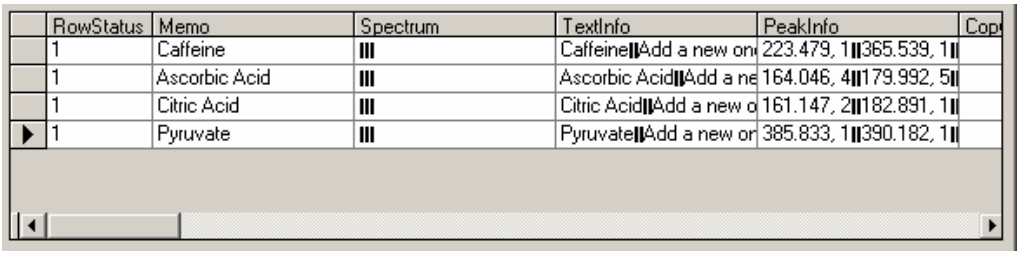


The Select Library window appears. Highlight the library where the spectrum is going to be added, and click *OK*.



The spectrum is now added to the library. On the top right section of the SpectrumSearch interface, the newly added spectrum is displayed as the only entry in the library list, and only a *RowStatus* column is shown.

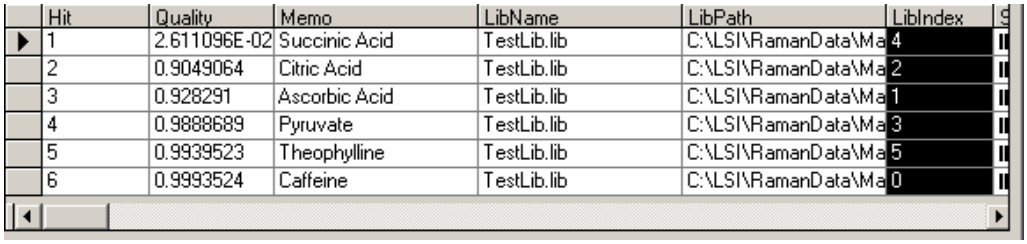
To confirm that the newly added spectrum is in the library, go to *Library Management->View Library*, highlight the library path/name, and click *Ok* in the Select Library window. The newly added library spectrum is shown as the last entry (row), after other entries for spectra that are already in the library.



	RowStatus	Memo	Spectrum	TextInfo	PeakInfo	Cop
	1	Caffeine		Caffeine||Add a new on	223.479, 1||365.539, 1|	
	1	Ascorbic Acid		Ascorbic Acid||Add a ne	164.046, 4||179.992, 5|	
	1	Citric Acid		Citric Acid||Add a new o	161.147, 2||182.891, 1|	
▶	1	Pyruvate		Pyruvate||Add a new or	385.833, 1||390.182, 1|	

Delete a Spectrum

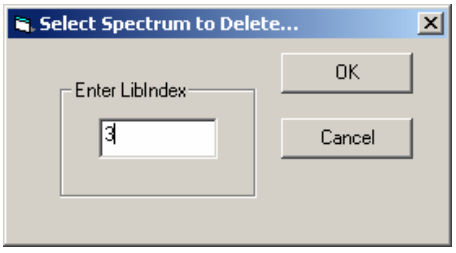
This allows for deleting a spectrum from a library. Deleting a spectrum from a library requires knowing the "LibIndex" number for this spectrum in the library. *LibIndex* is a sequential integer number (starting from 0) assigned to the spectrum when it is first added to the library. This number can be found when the library is being searched, as shown in the highlighted *LibIndex* column in the below figure.



	Hit	Quality	Memo	LibName	LibPath	LibIndex
▶	1	2.611096E-02	Succinic Acid	TestLib.lib	C:\LSI\RamanData\Ma	4
	2	0.9049064	Citric Acid	TestLib.lib	C:\LSI\RamanData\Ma	2
	3	0.928291	Ascorbic Acid	TestLib.lib	C:\LSI\RamanData\Ma	1
	4	0.9888689	Pyruvate	TestLib.lib	C:\LSI\RamanData\Ma	3
	5	0.9939523	Theophylline	TestLib.lib	C:\LSI\RamanData\Ma	5
	6	0.9993524	Caffeine	TestLib.lib	C:\LSI\RamanData\Ma	0

Once the LibIndex for the spectrum is known, click on *Library Management->Delete a Spectrum* to bring up the Select Library window, highlight the library in which the spectrum is going to be deleted. Click *OK*.

The Select Spectrum to Delete window appears. Enter the *LibIndex* number associated with that spectrum in the textbox.

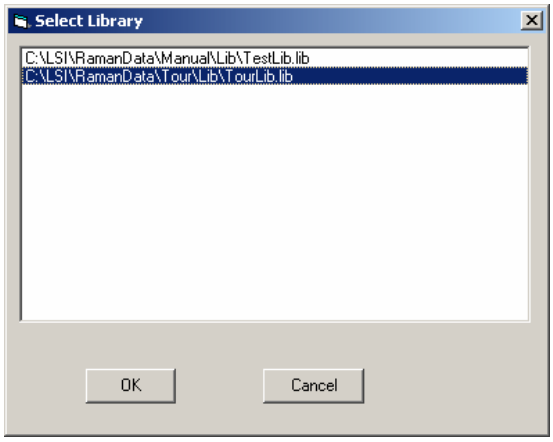


Click *OK*. The spectrum is then deleted. To confirm, go to *Library Management->View Library*, highlight the library path/name, click *OK*. The deleted spectrum row is empty except showing a *RowStatus* = 4 (which means "Deleted").

	RowStatus	Memo	Spectrum	TextInfo	PeakInfo	Cop
	1	Caffeine		Caffeine||Add a new on	223.479, 1||365.539, 1|	
	1	Ascorbic Acid		Ascorbic Acid||Add a ne	164.046, 4||179.992, 5|	
	1	Citric Acid		Citric Acid||Add a new o	161.147, 2||182.891, 1|	
	4					
	1	Succinic Acid		Succinic Acid||Add a ne	268.417, 1||274.215, 1|	
▶	1	Theophylline		Theophylline||Add a nev	164.046, 1||208.984, 2|	

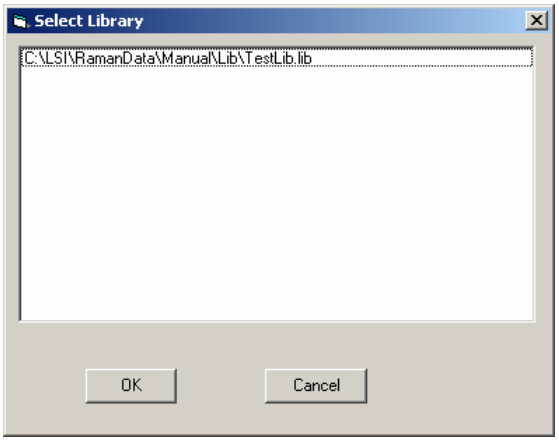
Delete Library from List

This allows for deleting a library from the library list. To do this, click *Library Management->Delete Library from List.* The Select Library window appears, which lists all current libraries on the library list. Highlight the library path/name from the Select Library window.



Click *Ok*, the library selected is then removed from the library list.

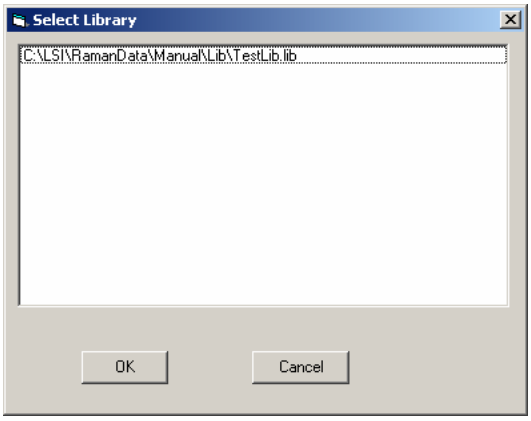
To confirm that the library is removed from the library list, go to *Library Management->View Library*. The library that was deleted from the library list is now not listed in the Select Library window.



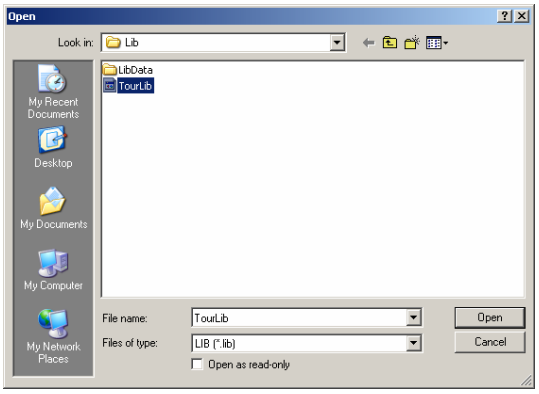
Note that once a library is deleted from the library list it will not be used in future library search sessions unless it is added back onto the library list again.

Add Library to List

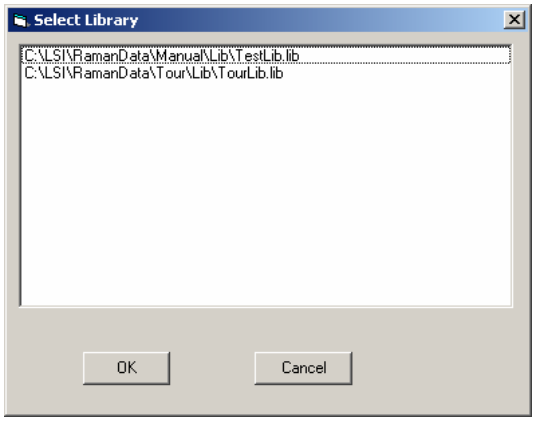
This allows for adding a library to the library list. To do this, first make sure that the library is not on the library list yet by going to *Library Management->View Library* and inspecting whether the library to be added has already been listed in the Select Library window.



If not, click *Library Management->Add Library to List,* browse to the directory where the library is located. Select the library name, and click *OK*.



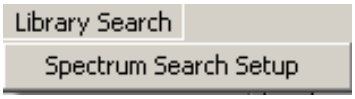
To confirm that the library is added to the library list, go to *Library Management -> View Library*. The library that was added to the library list is now listed in the Select Library window.



Note: Do not add an empty library to the library list. Empty libraries may cause SpectrumSearch to crash when a search is commenced.

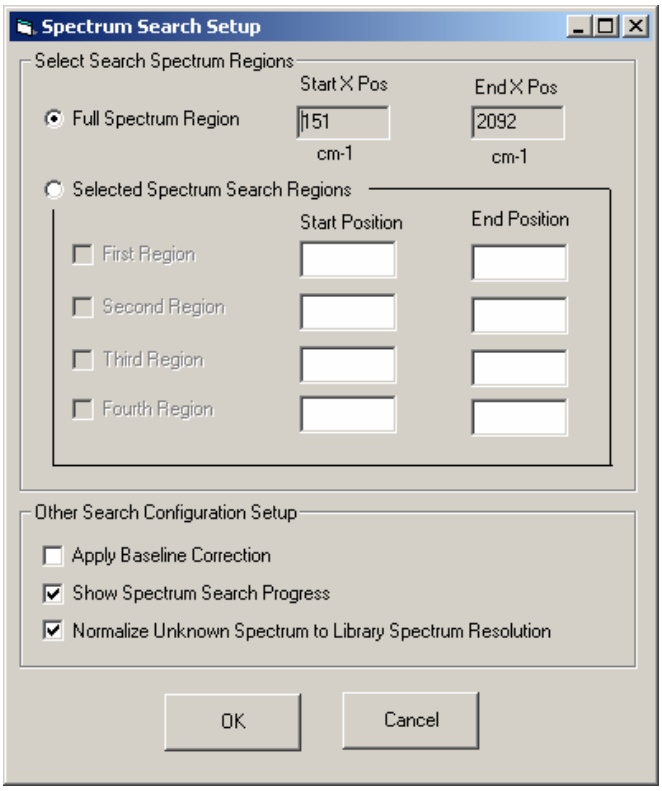
The Library Search Menu

The Library Search menu contains the *Spectrum Search Setup* menu item.



Spectrum Search Setup

The first step in performing spectrum search is to configure the search parameters. These parameters operate on all the libraries that have been chosen (i.e., that are added to the library list) for the search session being carried out.



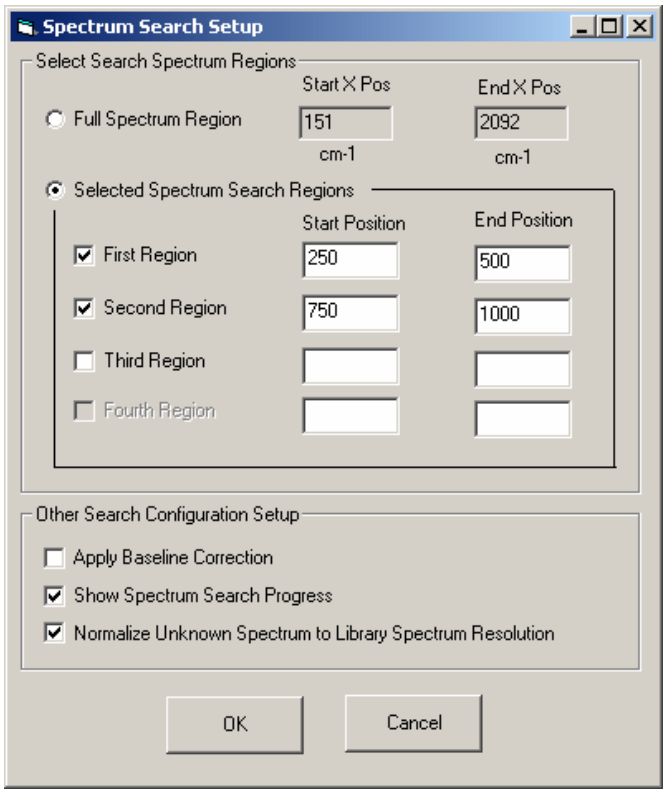
Note that the spectrum search parameters are saved in the configuration file (the same configuration file that the user has selected to use when RamanSoft is first started, for example, Default.rcf), and they need to be changed, if necessary, when a new search session is commenced. Such change is particularly important if a very restricted region or spectral feature had been chosen in the previous search session.

Select Search Spectrum Regions

This allows for choosing the full spectrum range or selecting a region or regions of interest within the full spectral range to perform the library search.

The *Full Spectrum Region* allows for searching the library using the entire spectral range. The *Start X Pos* and *End X Pos* fields display the start wavenumber and end wavenumber of the spectral range of the library. These are the same numbers as the *Start Position* and *End Position* in the Library Creation Configuration Setup window.

Selected Spectrum Search Regions allows for searching the library by user-chosen sub-regions. Up to four regions can be specified and used for a search session. The textbox fields of each region are dynamically available when the previous region is selected.



The numbers displayed in the textbox of the *Start Position* and *End Position* fields are those saved in the configuration file and used in the previous search session, and they may need to be modified for the current search session.

Other Search Configuration Setup

Apply Baseline Correction is to use GRAM/AI's Auto Level Baseline Correction algorithm. This method fits a best straight baseline to the non-peak areas of data. Thus it can only remove a linear (slope) background, and cannot remove more complex background profiles.
We recommend that if the library spectra were acquired using the RamanSoft automatic background removal feature, then this feature also be used in the search session, and that *Apply Baseline Correction* be not selected.

Show Spectrum Search Progress enables the display of the searching progress.

Normalize the Unknown Spectrum to Library Spectrum Resolution allows for normalizing the unknown spectrum intensity to the library spectrum resolution (8 bit, 16 bit, or 32 bit) set in the Library Creation Configuration Setup window. If this option is not chosen, the unknown spectrum is then displayed with its original intensity values.

Acquiring a Spectrum for Library Creation

The spectra used to create a library can be acquired from RamanSoft main interface or from the SpectrumSearch interface. If acquired from the RamanSoft main interface, the user needs to make sure that the “GRAMS Format (*.spc)” checkbox is checked in the File Save Preferences window, accessed through “File”->”Preferences”. If acquired from the SpectrumSearch interface, the user needs to set the acquisition parameters from the RamanSoft main menu. This section describes how to acquire spectrum from the SpectrumSearch interface for library creation.

1. Set up acquisition parameters from RamanSoft main menu:

    a. Set up CCD integration time and other CCD camera parameters through *System -> CCD Configuration*.

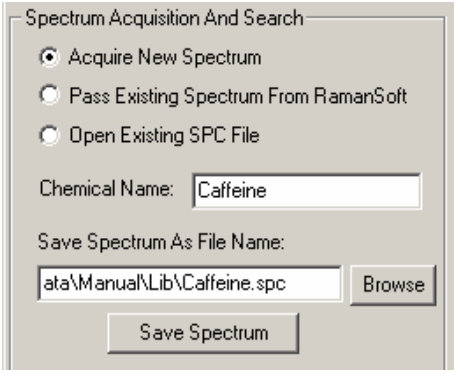
    b. Set up *Frames Per Measurement*, *Sample Name*, *Sample Info*, *File Prefix*, and file *Path* through *Acquisition->Setup*. Note that even though the files saved here are not used for library creation, it is recommended that these files be saved so that the spectra can be retrieved and displayed in RamanSoft for future reference.

    c. If automated data processing methods are desired, set them through *System->Automation->Setup*.

    d. Configure data processing algorithms through *Analysis->Algorithms Setup* for the chosen data processing methods in c. Remember to click *Apply* button on the Algorithm Setup page after an algorithm is configured.

    Note: If no data processing methods is chosen in the System Automation Setup window, the user needs to make sure that *System->Automation->On* is checked so that the acquired data can be passed to the SpectrumSearch interface.

2. In *Spectrum Acquisition and Search* group box, choose *Acquire New Spectrum*.

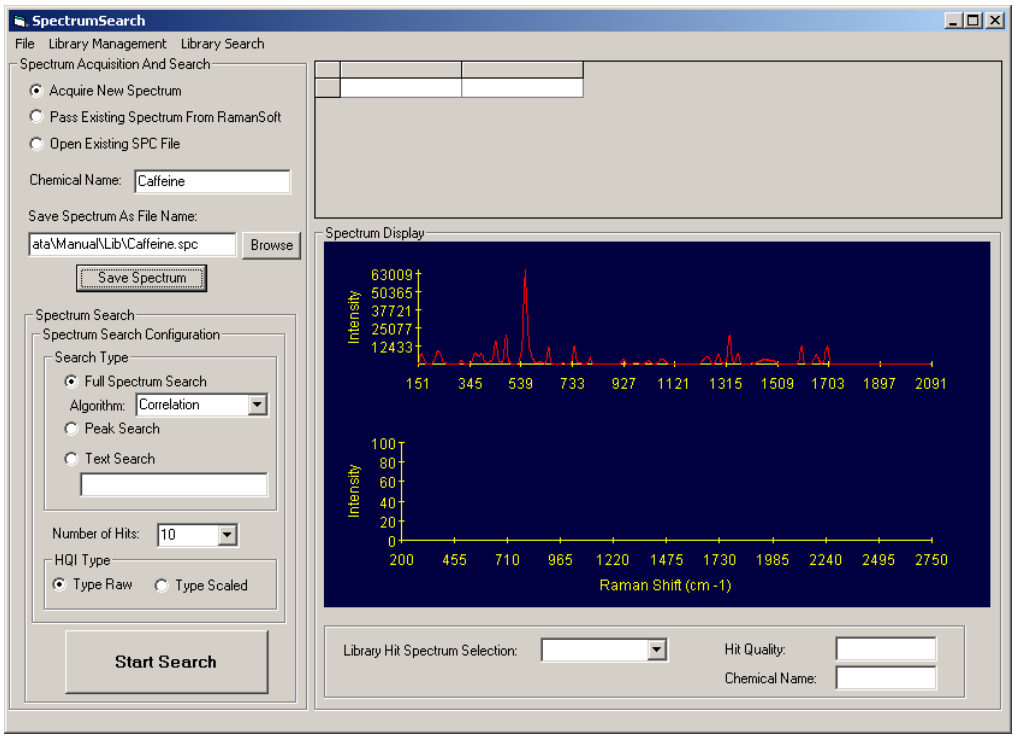


3. In the *Chemical Name* field, enter the name of the sample. Be sure to enter a meaningful name, as this is the name of the sample that is to be stored in the *Memo* field in the library when the spectrum is added to the library. This field is also retrieved from the library when a search is performed.

4. In the *Save Spectrum As File Name* field, enter the file name and path where the library spectrum is to be saved. The user can use the *Browse* button to browse to an existing directory/filename so that the file path/name is conveniently populated to save some typing.

5. Put the sample in the sample cell. If the External Sampling Module is used, make sure to close the lid.

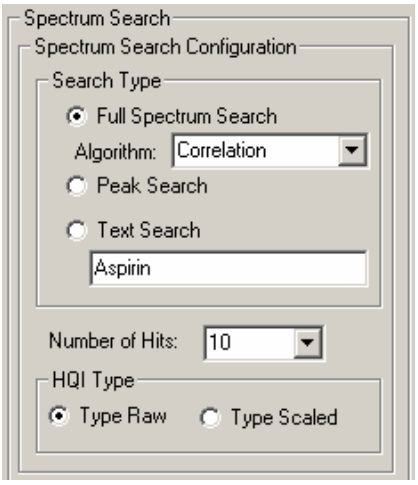
6. Click *Save Spectrum*. The spectrum is acquired, displayed in the upper panel of the *Spectrum Display* group box of SpectrumSearch interface, and saved.



Note that the acquired spectrum is also displayed in the RamanSoft Acquisition Window and Analysis window, and saved according to settings in step 1b.

Spectrum Search Configuration

There are three search types for performing a spectrum search: *Full Spectrum Search, Peak Search*, and *Text Search*. The default search type is *Full Spectrum Search*.

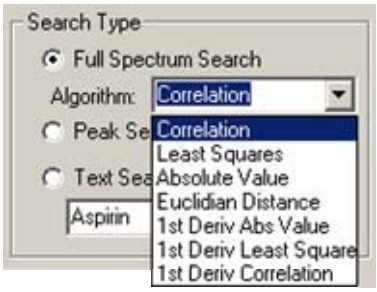


The *Number of Hits* displays the number of library matches to be displayed on the upper right panel of the SpectrumSearch interface. The default number is 10, but the user can choose between 1 and 40.

The *HQI Type* is the "Hit Quality Index" for the matched library items. It has two scoring schemes, *Type Raw* and *Type Scaled* for both full spectrum search and peak search. For text search, no scores are reported.

Full Spectrum Search

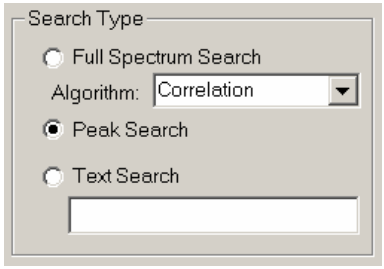
Seven algorithms are available for *Full Spectrum Search*: *Correlation*, *Least Squares*, *Absolute Value*, *Euclidian Distance*, *1st Derivative Absolute Value*, *1st Derivative Least Squares*, *and 1st Derivate Correlation*. Please refer to Spectral ID® User's Guide from Thermo Electron Corporation for more details on these algorithms. The default algorithm is the Correlation Algorithm. It gives better search results in most cases.



Under the *Type Raw* scoring scheme, a number close to "0" means a good "Hit Quality" and a number close to "1" (or "1.414", depending on the algorithm used) means a poor hit for full spectrum search algorithms. Under the *Type Scaled* scoring scheme the opposite is true.

Peak Search

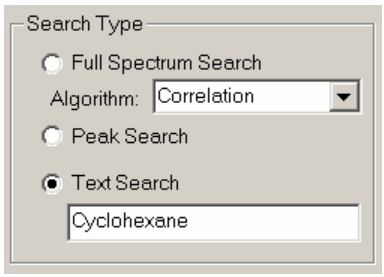
The *Peak Search* uses a peak matching algorithm to identify library matches. A peak table is created from the unknown spectrum and compared to the peak table for each entry stored in the library. The default peak search algorithm is the single side peak search where the peaks of the unknown sample spectrum are compared to the peaks in the library spectra with only the ascending side of the peak. The user can choose *Peak Search Double Side* in the Library Creation Configuration Setup window so that the unknown spectrum peaks are compared with both the ascending side and the descending side of the library spectra peaks. Double side peak search should give more accurate search results.



Under the *Type Raw* scoring scheme, a number closer to “100” means a good hit quality and a number closer to “0” means a poor match for peak search. Under the *Type Scaled* scoring system, a number closer to “-100” indicates a good match, and a number closer to “0” indicates a poor match.

Text Search

Text Search can be used to search each library entry by what is stored in its memo field. This search mode does not require an unknown spectrum to be loaded. It is used to quickly locate a library spectrum using the information in the memo field of the library.



Acquiring/Loading Spectrum for Library Searching

There are three spectrum search modes: acquiring a new spectrum, opening an existing spectrum from RamanSoft, or opening an existing SPC file to perform library searching.

Acquiring a New Spectrum

This is the RamanSoft “on-line” search mode. To acquire and search an unknown spectrum in one-step, proceed with the following procedure, most of which are the same as that for “Acquire Spectrum for Library Creation”. To achieve better search results, it is recommended that the acquisition parameters and configuration settings are as identical to those used for acquiring spectrum for library creation as possible.

1. Set up acquisition parameters from RamanSoft main menu:

    a. Set up CCD integration time and other CCD camera parameters through *System -> CCD Configuration*.

    b. Set up *Frames Per Measurement*, *Sample Name*, *Sample Info*, *File Prefix*, and file *Path* through *Acquisition->Setup*. Note that the files saved here are not used for

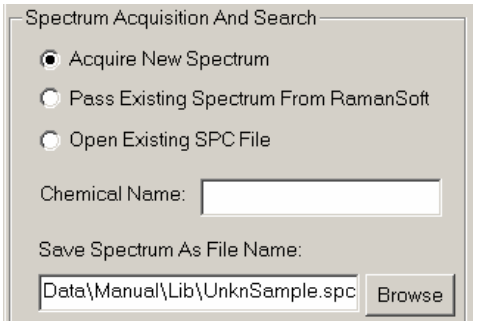
library search, but the user can opt to save it so that the spectra can be retrieved and displayed in RamanSoft for future reference.

c. If automated data processing methods are desired, set them through *System -> Automation->Setup*.

d. Configure data processing algorithms through *Analysis->Algorithms Setup* for the methods chosen in c. Remember to click *Apply* button on the Algorithm Setup page after an algorithm is configured.

> **Note**: If no data processing methods is chosen in the System Automation Setup window, the user needs to make sure that *System->Automation->On* is checked so that the acquired data can be passed to the SpectrumSearch interface.

2. In *Spectrum Acquisition and Search* group box, choose *Acquire New Spectrum*.

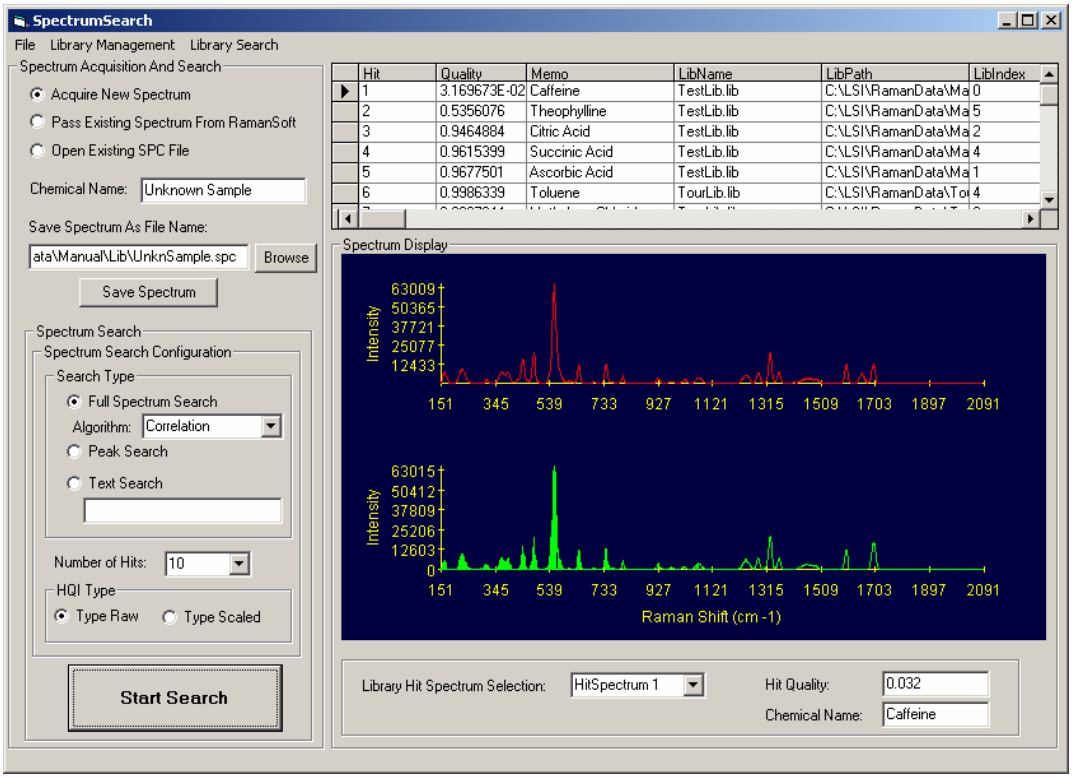


3. In the *Save Spectrum As File Name* field, enter the file name (e.g., UnknSample.spc) and path where the acquired unknown sample spectrum (in SPC format) is to be saved.

4. Put the sample in the sample cell. If the External Sampling Module is used, make sure to close the lid.

5. Set search configurations (see Sections “The Library Search Menu” and “Spectrum Search Configuration”).

6. Press *Start Search* button. After a transit window disappears, the unknown sample spectrum is acquired, displayed in the Acquisition Window and Analysis Window, and in the upper plot (in red) of the *Spectrum Display* panel of SpectrumSearch interface. The best-matched library spectrum is displayed (in green) in the lower plot.



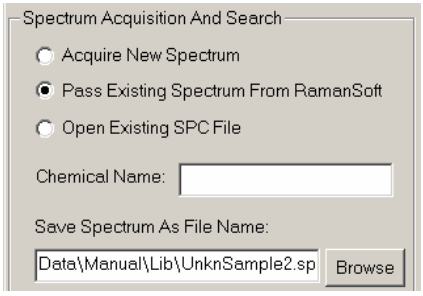
7. The search results are saved in a text file with the same filename as the spectrum (SPC) file (e.g., UnknSample.txt).

Passing an Existing Spectrum from RamanSoft

This is for searching a spectrum that has previously been acquired and saved in RamanSoft (files with *.raw or *.pro extensions), or a spectrum that was acquired and displayed in the Analysis Window.

1. If a spectrum has not been displayed on the Analysis Window yet, go to *File->Open* to open an existing spectrum and display it the Analysis Window.

2. Check the *Pass Spectrum from RamanSoft* radio button.



3. In the *Save Spectrum As File Name* field, enter the file name (e.g., UnknSample2.spc) and path where you want the spectrum to be saved by the Spectrumsearch module. The user can use the *Browse* button to browse to an existing directory/filename.

4. Set search configurations (see Sections “The Library Search Menu” and “Spectrum Search Configuration”).

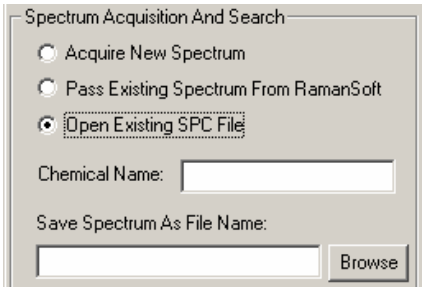
5. Press *Start Search*. After a transit window disappears, the search results are displayed in the SpectrumSearch interface.

6. The search results are saved (as a text file) with the same filename as that had been entered in the *Save Spectrum as File Name* field (e.g., UnknSample2.txt).

Opening an Existing SPC File

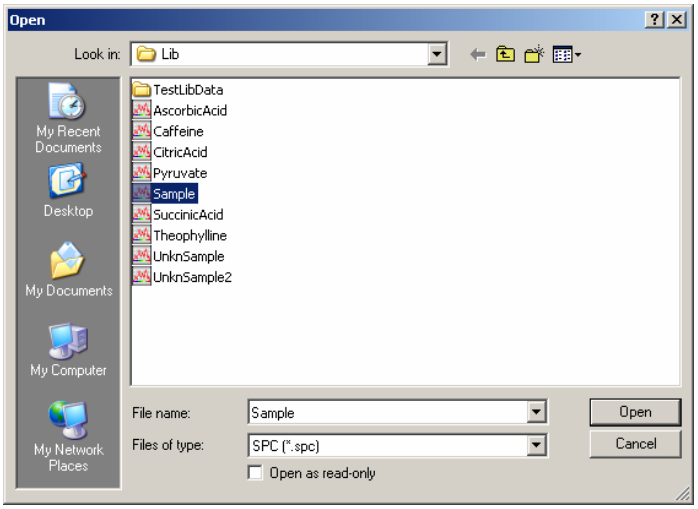
The SPC file can be saved from either RamanSoft main interface or from SpectrumSearch interface.

1. Check the *Open Existing SPC File* radio button.



2. Set search configurations (see Sections “The Library Search Menu” and “Spectrum Search Configuration”).

3. Press *Start Search*. Browse to select the SPC file to be searched.



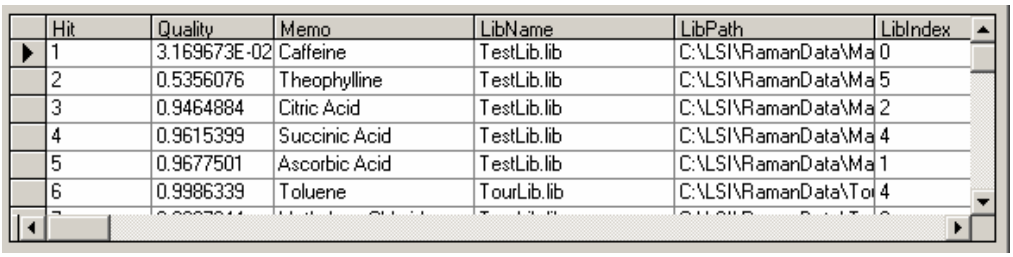
4. Click *Open*. The search starts. After a transit window disappears, the results are displayed in the SpectrumSearch interface.

5. The search results are saved as a text file with the same filename as the name of the spectrum being searched (e.g., sample.txt).

Note that in this search mode, SpectrumSearch does not save an additional SPC file for the unknown sample spectrum during the search session.

Viewing Search Results

On the SpectrumSearch interface, the top right section lists the matched (Hit) library items in a decreasing “goodness-of-match” order. More information about each hit item can be viewed by scrolling to the right of the window.



Hit	Quality	Memo	LibName	LibPath	LibIndex
1	3.169673E-02	Caffeine	TestLib.lib	C:\LSI\RamanData\Ma	0
2	0.5356076	Theophylline	TestLib.lib	C:\LSI\RamanData\Ma	5
3	0.9464884	Citric Acid	TestLib.lib	C:\LSI\RamanData\Ma	2
4	0.9615399	Succinic Acid	TestLib.lib	C:\LSI\RamanData\Ma	4
5	0.9677501	Ascorbic Acid	TestLib.lib	C:\LSI\RamanData\Ma	1
6	0.9986339	Toluene	TourLib.lib	C:\LSI\RamanData\To	4

Hit An integer number for the matched library item ordered in decreasing “goodness-of-match” according to the hit quality listed in the *Quality* column. “1” indicates the best matched item.

Quality A numerical number for the hit score, which depends on the search type and HQI type. In the *Type Raw* scoring scheme for full spectrum search, this number ranges from 0 to 1 or 1.414; the lower the score, the better the match (as shown in the above figure).

Memo The chemical name of the sample that the user puts in the *Chemical Name* field of the *Spectrum Acquisition and Search* group box of the SpectrumSearch interface when acquiring a spectrum for library creation.

LibName The name of the library that the spectrum belongs to.

LibPath The file path of the library.

LibIndex The index number for the library spectrum. This number is the sequence number when the spectrum is first added to the library. The first item added has a LibIndex number “0”, the second item “1”, and so on.

Spectrum The actual spectrum of the library item. Not displayed in SpectrumSearch.

TextInfo The comment field (i.e., *Memo* field) for the library item and other information such as how the spectrum is added to the library (e.g., by Adding New Spectrum).

PeakInfo The peak table for the library item. The peak table is a table of values where each entry consists of the X axis value of a local maximum in the spectral data followed by the relative intensity of the peak on a scale of 0 to 9. Entries are separated by the symbol “**||”.**

CopyRight The copyright of the library spectrum if it is from a commercial database. This column is empty in SpectrumSearch.

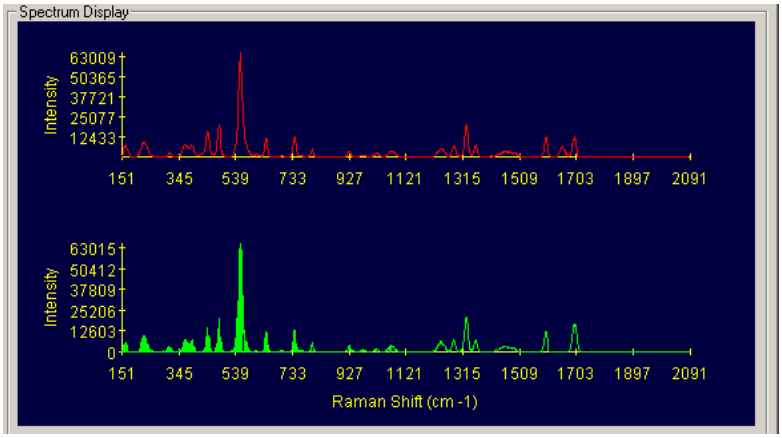
MolFile The filename for the molecular structure of the sample that produced the library spectrum. This column is empty in SpectrumSearch.

RecType The record type. “1” indicates valid record type.

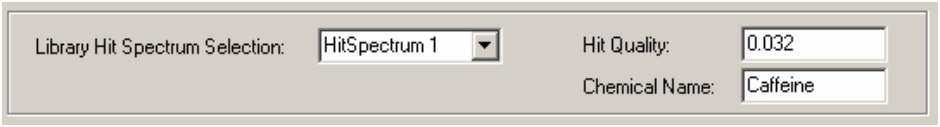
ErrNum The error number for the library item.

ErrDescription The error description for the *ErrNum*.

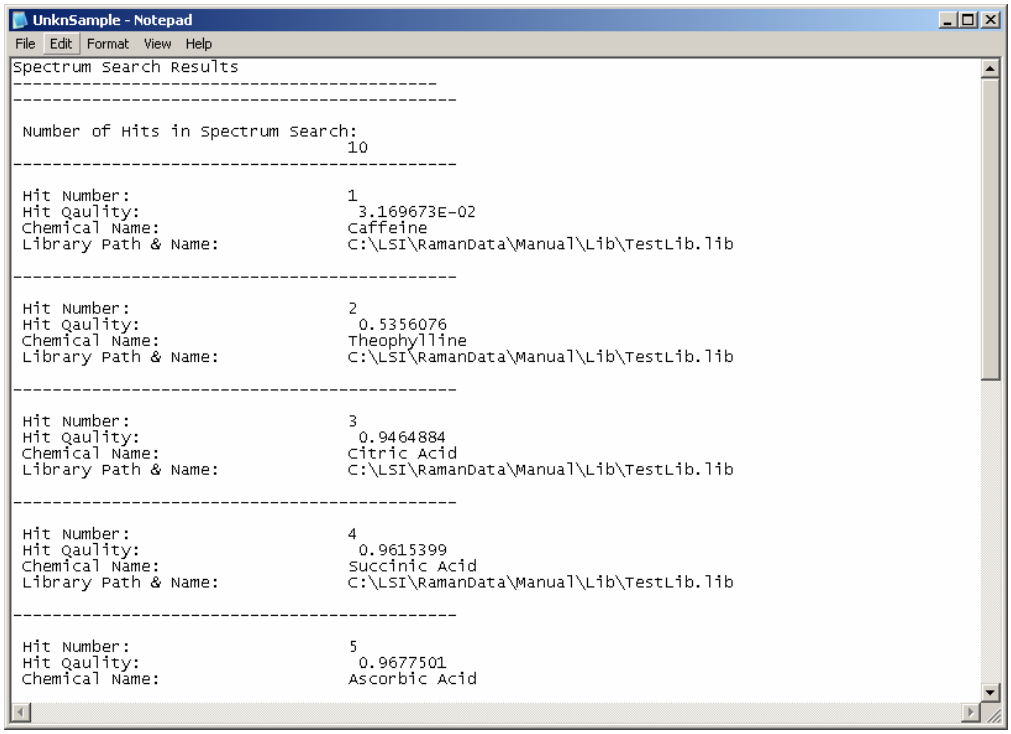
The *Spectrum Display* panel displays the unknown sample spectrum (top plot, red) and the spectrum of the best matched library spectrum (lower plot, green).



Other matched library spectrum can be viewed on the lower plot of the Spectrum Display panel through the *Library Hit Spectrum Selection* pull-down selection menu. The *Hit Quality*(i.e., the score), and the *Chemical Name* (i.e., the memo field) of the selected library entry are also displayed.



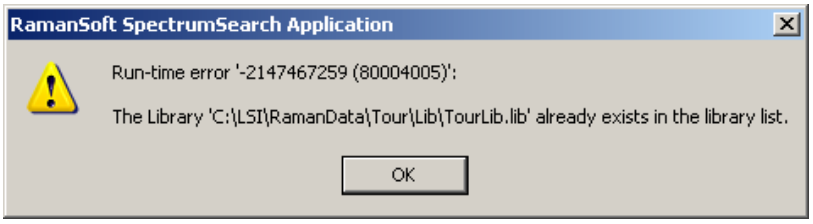
The saved search results (in a text file) contain the Hit Number, Hit Quality, Chemical Name, and Library Path & Name of all the library hit items configured in the *Number of Hits* field in the SpectrumSearch interface.



SpectrumSearch Troubleshooting

Library Already in the Library List

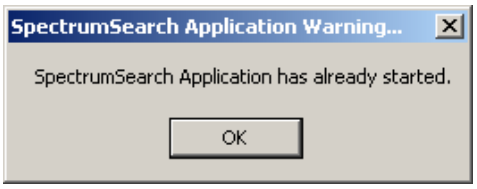
If a library to be added to the library list is already on the library list, the following window will appear:



Click *OK* to exit the SpectrumSearch program, and proceed to the following section “Restarting SpectrumSearch Application”.

SpectrumSearch Application Already Started

If the SpectrumSearch application is already started, and the user clicks on RamanSoft *Tools* -> *SpectrumSearch* or the SpectrumSearch icon  on the Function Toolbar, the following warning window will appear:



Click *Ok*, and locate the current SpectrumSearch application window.

Two SpectrumSearch Application Interfaces

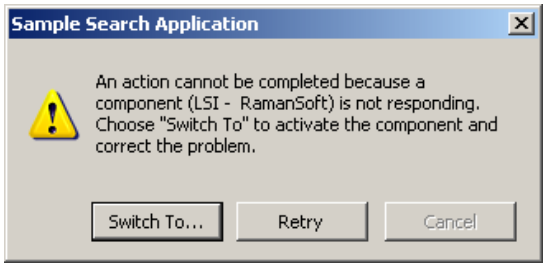
This might occur if the user has clicked *OK* on the SpectrumSearch Application Warning window (see above topic “SpectrumSearch Application Has Already Started”) and then proceeded to restart SpectrumSearch application (see “Restarting the SpectrumSearch Application” in this section). In this case, the user can launch a second SpectrumSearch interface without being warned again. Close the newly started SpectrumSearch application.

No Action Occurred After Pressing *Save Spectrum* or *Start Search*

The laser might be off, as the system automatically turns the laser off if there is no acquisition action for 30 minutes. Click on *System->Laser Setting->Laser On* to turn the laser on from the RamanSoft main menu (see section “RamanSoft Trouble Shooting” in Chapter 5 for more details).

SpectrumSearch Application Warning

If the following window appears after pressing the *Save Spectrum* or *Start Search* button,

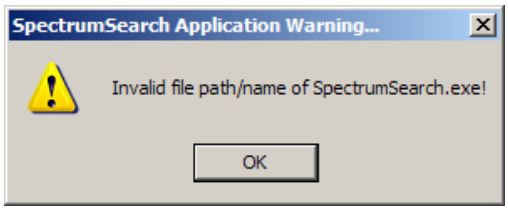


The laser might be off, as the system automatically turns the laser off if there is no acquisition for 30 minutes. Click on *Switch To*… button. The Laser Power Status Warning Window will appear.

Click on *Yes* to turn the laser on. Wait for the laser power to stabilize (the progress bar of the Laser Power Stabilizing Progress Window reaches the end). Click *Save Spectrum* or *Start Search* to continue.

Invalid File Path/Name of SpectrumSearch Executable

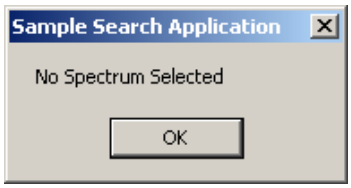
If the invalid file path/name of SpectrumSearch.exe occurs,



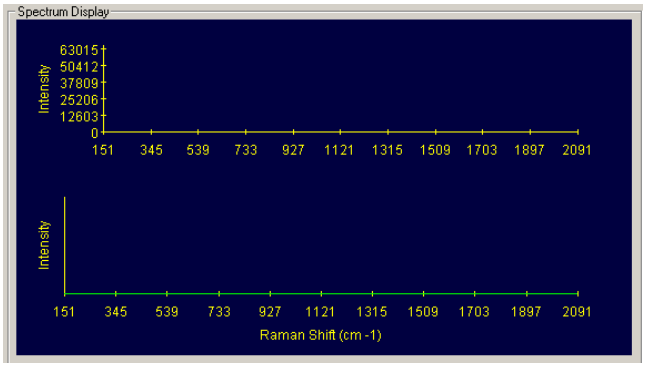
The SpectrumSearch executable might be relocated from its default location in C:\LSI\RamanSoft\exe\SpectrumSearch.exe. Proceed to “Restarting Spectrum Search Application” and browse to locate SpectrumSearch.exe from the right folder.

No Spectrum Selected

If the search mode is *Open SPC File* and the user clicked *Cancel* on the file Open window, the following warning window will appear:

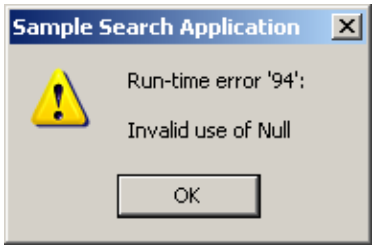


Click on *Ok*. The Spectrum Display window on the SpectrumSearch interface displays a green line on the horizontal axis of the lower plot with an un-scaled y-axis (to stand for an “empty” spectrum in the library). Re-select the right file and press *Start Search* to continue.



Run-time Error

If a run-time error of any type occurs, such as



The SpectrumSearch module needs to be restarted. Click *OK*, and proceed to the following subsection “Restarting the SpectrumSearch Application”.

Unknown Error Conditions

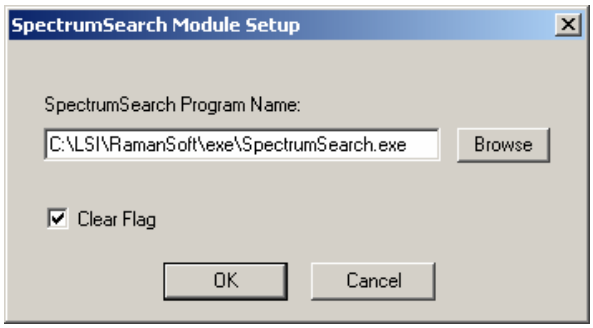
If the SpectrumSearch module crashes, or any other error conditions arise from this module, proceed with “Restarting the SpectrumSearch Application”.

Upon any other error conditions, exit both programs (exit SpectrumSearch first and then exit RamanSoft), and re-launch RamanSoft.

If neither of the above two procedures works, restart the computer.

Restarting the SpectrumSearch Application

Go to RamanSoft System->SpectrumSearch, check the *Clear Flag* checkbox.



Click *OK*, and then restart the SpectrumSearch module by clicking on RamanSoft *Tools* -> *SpectrumSearch* or the SpectrumSearch icon  on the Function Toolbar

7 The SpectrumPredict Module

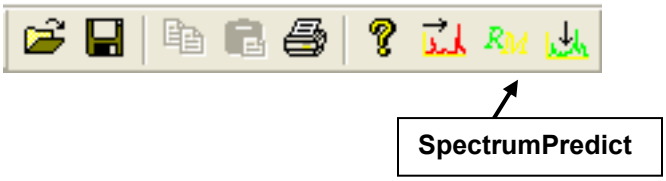
Overview

The SpectrumPredict module is an integration of RamanSoft with the Thermo Electron Corporation's IQ Predict™ for seamlessly performing sample spectrum acquisition and sample prediction using multivariate Chemometric methods. This includes both qualitative methods like Principal Component Analysis (PCA), Discriminant Analysis for quality control/assurance application, and quantitative methods like Principal Component Regression (PCR), Partial Least Squares (PLS) analysis for quantitative concentration prediction applications. Users need to have Thermo Electron Corporation's GRAMS/AI™ with PLSplus IQ™ add-on to create calibration files; the RamanSoft SpectrumPredict interface allows for using these calibration files to perform spectrum prediction online or offline. Please refer to "PLSplus IQ™ User Guide" for a thorough introduction to Spectroscopic Qualitative Analysis and Spectroscopic Quantitative Analysis. This manual assumes a working knowledge of the Chemometrics methods introduced in the "PLSplus IQ™ User Guide".

Launching the SpectrumPredict Application

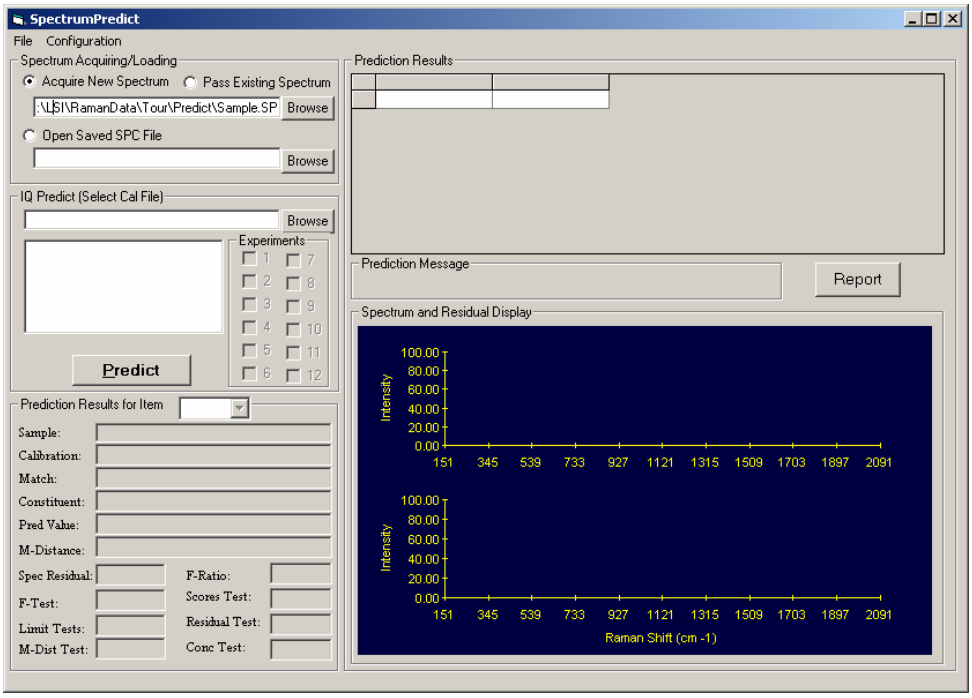
The SpectrumPredict module is launched through *Tools->SpectrumPredict* from RamanSoft main menu.

The module can alternatively be launched by clicking on the SpectrumPredict icon on the System Toolbar.



The SpectrumPredict Interface

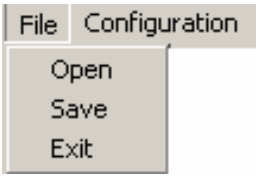
The main interface for the SpectrumPredict module appears as follows:



The main menu of the SpectrumPredict interface consists of *File* and *Configuration* menu items.

The File Menu

The file menu consists of *Open*, *Save*, and *Exit* menu items:



Open

This opens a previously saved prediction result file in either RTF format or Text format, and displays the prediction results in the SpectrumPredict Report window.

Save

This allows the user to save the prediction results into a RTF file or a plain text file directly from the File menu after the results are obtained in a SpectrumPredict session.

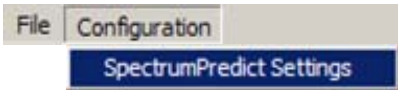
The alternative way to save the prediction results is to click on the *Report* button on the SpectrumPredict interface, and press the *Save* button on the SpectrumPredict Report window. See section “View Prediction Results” for more details.

Exit

This exits the SpectrumPredict module.

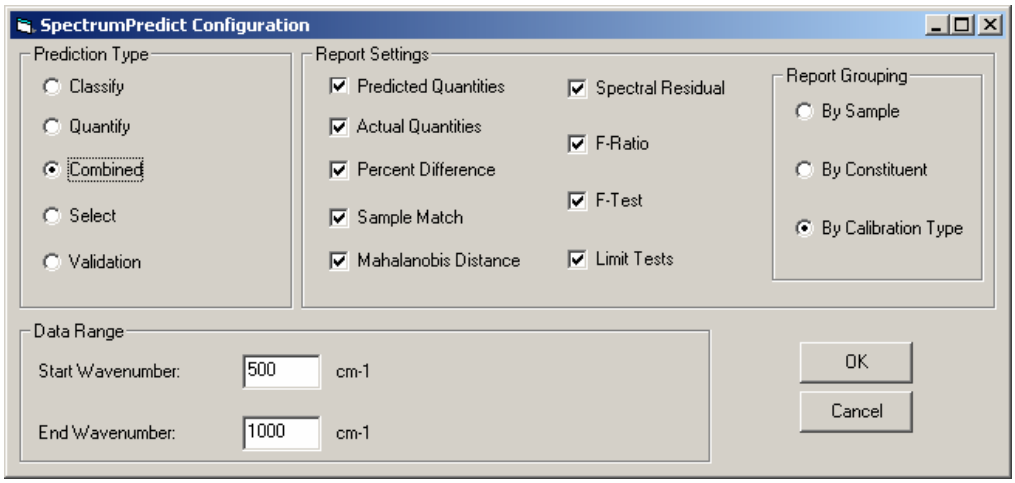
The Configuration Menu

This menu configures the parameters for spectrum prediction.



SpectrumPredict Settings

The SpectrumPredict Configuration window allows user to configure *Prediction Type*, *Report Settings* for the report file, and specify the *Data Ran*ge for spectrum and residual display on the SpectumPredict interface.



Prediction Type

You may choose from *Classify*, *Quantify*, *Combined*, or *Select*. The type you choose determines how the calibration models are used when predicting unknowns.

Classify

If the prediction type is set to *Classify*, only discriminant analysis is performed on all of the selected calibration models. For PCA, PCR, PLS-2, or Discriminate models, a single Mahalanobis distance value will be returned for each calibration model selected along with the Pass/Fail results determined from the model. For PLS-1, a Mahalanobis distance will be returned for each component since PLS-1 builds a separate model for each component.

Quantify

In this mode, quantitative results will be reported on all of the selected calibration models. If the one selected is a PCA or discriminate model, only the distance statistics will be reported (since there is no quantitative information in these models).

Combined

This mode first uses classification (Discriminant Analysis) to determine if the unknown is within the selected model(s) specified by the Mahalanobis distance. If so, it will calculate the quantities and report them. If not, quantitative information is not reported for that model. This allows a calibration file to be built with numerous models which will automatically select the correct model to use (if any) and report the results.

Select

This mode performs the predictions of spectra against the selected calibrations, but only reports the results for those calibrations that pass the Mahalanobis Distance test (either Pass (P) or Possible (?)). This allows the user to select multiple calibrations and let the prediction program figure out which calibrations best match the sample. Be aware that in some cases, none of the calibrations may match and an empty report will result.

Validation

This mode performs unknown sample validation. The following parameters must match for a sample to be considered "valid" for use with a given calibration: data point spacing, first X value (must be on or outside the calibration limit), last X value (must be on or outside the calibration limit), X and Y axis units.

If the sample is validated, spectrum prediction is then performed on the sample using the same statistics as those of the *Combined* prediction type.

Report Settings

The report settings allow for selecting the report format and the various statistics to use for generating the report.

Predicted Quantities

Select whether to report the predicted quantities (for quantitative models). Normally, quantities will always be reported when you set the prediction type to *Quantify*. You can use this option to exclude quantities from the report, and only the selected statistics will be reported.

Actual Quantities

Choose whether to report the actual quantities of the sample. This item would only appear in the report when the prediction type is set to *Validation*.

Percent Difference

Select whether to report the percent difference between the predicted quantities and the actual quantities. This item would only appear in the report when the prediction type is set to *Validation*.

Sample Match

Choose whether to report the result of match/mismatch between the model and the sample.

Mahalanobis Distance

Select whether to report the Mahalanobis Distance metric.

Spectral Residual

Choose whether to report the value of the spectral residual.

F-Ratio

Choose whether to report the F-Ratio values on the spectral residual.

F-Test

Choose whether to report the calculated F-Test values on the spectral residual.

Limit Tests

Choose whether to report the Pass/Fail tests on various statistics:

Scores	The calculated factor scores for the sample are checked to insure that they are within the range of scores of the training data set data for the model.
F-test	An F-test is applied to the spectral residual to check that it is statistically similar to the training set data for the model. Samples that have F-test values greater than 0.99 fail the test.
M. Distance	The calculated Mahalanobis Distance for the sample is compared to the *M Dist. Accept* and *M Dist. Reject* levels that were set when the calibration was saved (i.e., when the calibration file is created). Samples below the *M. Dist. Accept* level will pass. Sample between the two levels are given a “Possible” rating. Any samples above the *M. Dist. Reject* level will fail the test.
Concentration	The predicted concentration values, if applicable, are checked to insure that they are within the range of concentration for each constituent in the original training set for the selected model.

Report Grouping

Choose the grouping method to generate the report.

By Sample	This is to group the prediction results organized by sample (Note: this feature would be most useful if multiple spectra could be loaded for prediction in the same time. In SpectrumPredict, however, only one spectrum can be predicted at one time).
By Constituent	This is to group the prediction results organized by constituent. When a training data set is created, the constituents of the samples are specified. The corresponding constituents in the unknown sample are then predicted.
By Calibration type	This is to group the prediction results organized by calibration type. A calibration file (Cal file) contains experiments built from one of the five calibration types: PLS-1, PLS-2, PCR, PCA, and Discriminate.

Data Range

Start Wavenumber The start wavenumber for the sample spectrum and the spectral residual (i.e., the difference between the sample spectrum and the predicted spectrum) plot.

End Wavenumber The end wavenumber for the sample spectrum and the spectral residual plot.

Note that the data range is only used for displaying the spectrum and residual on the SpectrumPredict interface. If the user intends to use selected regions of the spectrum for prediction, the user needs to create a calibration file with the selected regions. Please refer to "PLSplus IQ™ User's Guide" for how to do so.

Performing a Spectrum Prediction

Loading a Calibration File

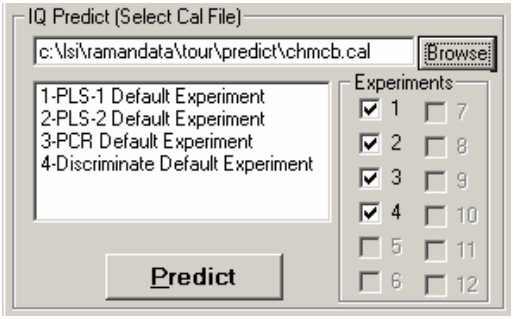
The calibration file is created using Thermo Electron Corporation's GRAMS/AI™ with PLSplus/IQ add-on. Please refer to the "Reference Guide" chapter of the "PLSplus IQ™ User's Guide" for creating a calibration file. Once the calibration models, or experiments, are set up in the calibration file, RamanSoft can load those experiments to perform on-line or off-line prediction of a sample spectrum.

A calibration file can contain several experiments, with each experiment containing one of the five calibration types: PLS-1, PLS-2, PCR, PCA, and Discriminate. These calibration types can be combined with other model building conditions (please refer to "PLSplus IQ™ User's Guide" for details) to build a specific experiment.

1. In the *IQ Predict (Select Cal File)* group box of the SpectrumPredict interface, click *Browse* button.

2. The Select Cal File Name window appears. Browse to locate the calibration file, and click *Open*.

3. The experiments created in the calibration file are then loaded in the text window under the *IQ Predict (Select Cal File)* group box. The user can choose any or all of the available experiments for prediction (here all four are chosen).

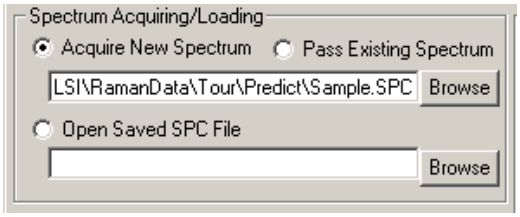


Acquiring/Loading a Spectrum for Prediction

There are three ways that the user can perform a spectrum prediction: the user can acquire a spectrum and perform prediction on line; the user can pass an existing spectrum displayed in the RamanSoft Analysis Window, or the user can pass a spectrum that was previously acquired and saved in the GRAMS SPC file format, to perform spectrum prediction off line.

Acquiring a New Spectrum

1. From the *Spectrum Acquiring/Loading* group box, choose *Acquire New Spectrum*. Type in the file path and name where the acquired spectrum is to be saved in SPC format from the SpectrumPredict interface (e.g., “Sample.SPC”). The user can also click the *Browse* button to conveniently browse to a desired directory to save the file.



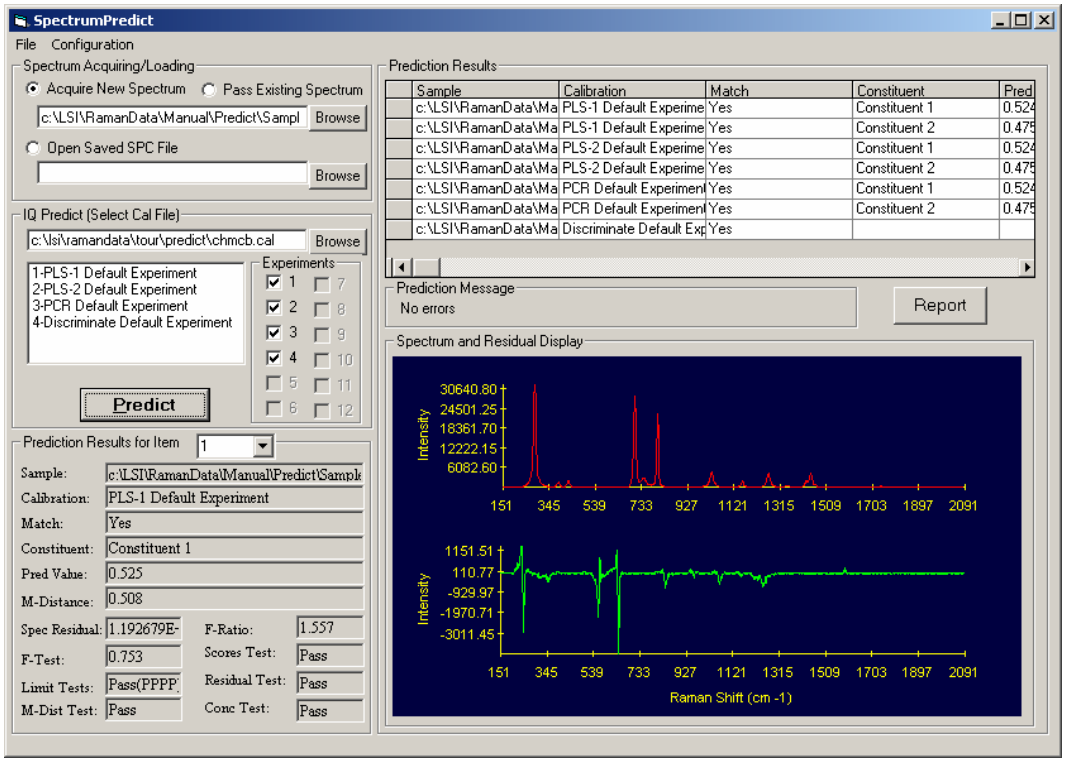
2. Set up acquisition parameters from RamanSoft main menu through the following menu items:
   a. *System->CCD Configuration*,
   b. *Acquisition->Setup*,
   c. *System->Automation->Setup*,
   d. If c, then *Analysis->Algorithms Setup*.

   The important parameters to set up are the CCD integration time, number of frames to average, background subtraction, or other data processing methods, if these methods were used to create the original training set (Note: it is recommended that that sample spectrum be acquired with as much the same experimental conditions with those for acquiring the training data set as possible). Input *Sample Name*, *Sample Info*, and *File Prefix* in the Acquisition Setup window. It is recommended that the files from RamanSoft and from SpectrumPredict interface be saved in the same directory for easier future reference.

   Note: If no data processing methods is chosen in the System Automation Setup window, the user needs to make sure that *System->Automation->On* is checked so that the raw spectrum can be displayed in the Analysis Window and passed to the SpectrumPredict interface.

3. Put the sample in the sample cell. Make sure that the laser is on by determining that *System -> Laser Setting->Laser O*n is checked. If not, click on *Laser On* to turn the laser on.

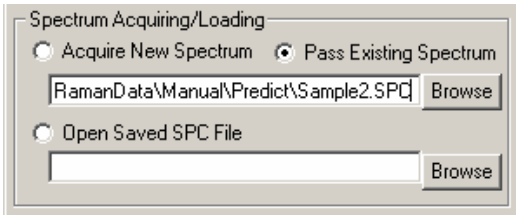
4. Press the *Predict* button. The spectrum is acquired and the prediction results are displayed on the SpectrumSearch interface. Note that the spectrum is also displayed on the Acquisition Window and Analysis Window as well.



Passing an Existing Spectrum

This option can be used on any spectrum displayed in the RamanSoft Analysis Window.

1. From *Spectrum Acquiring/Loading* group box, choose *Pass Existing Spectrum*. Type in the file path and name where you want the spectrum to be saved in SPC format from the SpectrumPredict interface (e.g., “Sample2.SPC”). The user can also click the *Browse* button to conveniently browse to a desired directory to save the file.



2. Open a spectrum file from RamanSoft and display it in the Analysis Window.

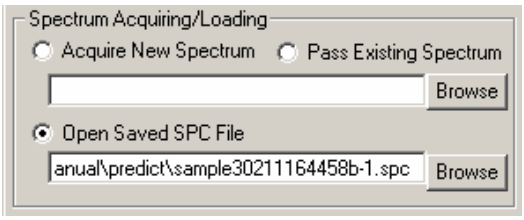
3. Press the *Predict* button. The spectrum and the prediction results are displayed on the SpectrumSearch interface.

Opening a Saved SPC File

1. From *Spectrum Acquiring/Loading* group box, choose *Open SPC File*.

2. Browse to locate the SPC file saved either through the SpectrumPredict interface or through RamanSoft (Note that in RamanSoft if “GRAMS Format (*.spc)” is checked in the File Save Preference window, the acquired spectrum will also be saved in SPC format in addition to the

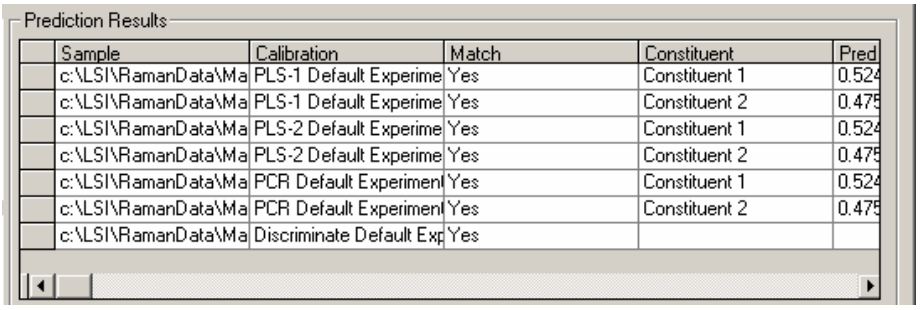
LSI format and the text format. We recommend that all spectra be saved in SPC as well in RamanSoft so that they will be readily available for all GRAMS functionalities).



3. Press the *Predict* button. The prediction results are displayed on the SpectrumSearch interface.

Viewing the Prediction Results

The prediction results are listed on the upper right section of the SpectrumPredict interface in a table form. Each row constitutes a prediction item. If a sample is composed of two constituents (Constituent 1, Constituent 2), there will be two line items (rows) for each experiment selected for quantitative predictions such as PLS-1, PLS-2, and PCR. For qualitative prediction method Discriminate Analysis, only one row is generated.



	Sample	Calibration	Match	Constituent	Pred
	c:\LSI\RamanData\Ma	PLS-1 Default Experime	Yes	Constituent 1	0.524
	c:\LSI\RamanData\Ma	PLS-1 Default Experime	Yes	Constituent 2	0.475
	c:\LSI\RamanData\Ma	PLS-2 Default Experime	Yes	Constituent 1	0.524
	c:\LSI\RamanData\Ma	PLS-2 Default Experime	Yes	Constituent 2	0.475
	c:\LSI\RamanData\Ma	PCR Default Experiment	Yes	Constituent 1	0.524
	c:\LSI\RamanData\Ma	PCR Default Experiment	Yes	Constituent 2	0.475
	c:\LSI\RamanData\Ma	Discriminate Default Exp	Yes		

More prediction results and statistics can be viewed by scrolling to the right of the window. Note that the columns list all available result information/statistics. These columns are not configurable (Note: the configurations set in the SpectrumPredict Configuration window are for the report file only, not for the prediction results as tabulated as above on the SpectrumPredict interface).

Sample The filename for the sample spectrum.

Calibration The calibration type (i.e., the experiment loaded from the Cal file)

Match Whether the unknown sample matches the calibration samples (Yes/No/Possible)

Constituent The constituents of the sample (defined when the training data file (.tdf file) is established)

Pred Value The predicted concentration value of the constituent

Actual Value The actual value of the constituent. For *Validation* calibration type only.

Percent Diff The percent difference between the *Pred Value* and the *Actual Value*. For *Validation* calibration type only.

M Distance The Mahalanobis Distance between the unknown sample and the calibration samples.

Limit_Tests This statistics contains four Pass/Fail tests: Scores, F-Test, M. Distance, Concentration.

Scores Test The calculated factor scores for the sample are checked to insure that they are within the range of scores of the training data set data for the model.

Residual Test The calculated residuals for the sample are checked to insure that they are within the range of residuals of the training data set data for the model.

M-Dist Test The calculated Mahalanobis Distance for the sample is compared to the *M Dist. Accept* and *M Dist. Reject* levels that were set when the calibration was saved (i.e., when the calibration file is created). Samples below the *M. Dist. Accept* level will pass. Sample between the two levels are given a “Possible” rating. Any samples above the *M. Dist. Reject* level will fail the test.

Conc Test The predicted concentration values, if applicable, are checked to insure that they are within the range of concentration for each constituent in the original training set for the selected model.

Spec Residual The summed spectral residual of the constituent.

F-Ratio The F-ratio value on the spectral residual

F-Test The F-test value on the spectral residual

Error Description of error if there is an error condition.

SampleSpectrum Sample spectrum. Not displayed in this column. It is displayed in the upper plot (in red) of the *Spectrum and Residual Display* group box.

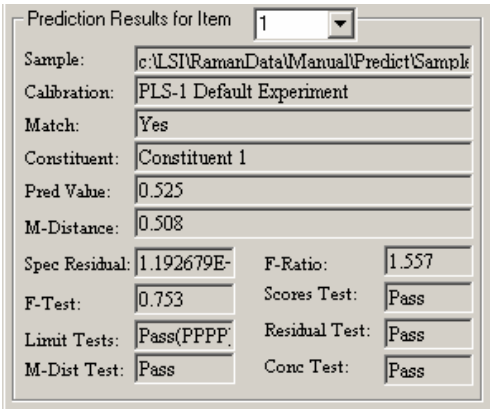
ResidualSpectrum Residual spectrum. Not displayed in this column. It is displayed in the lower plot (in green) of the *Spectrum and Residual Display* group box.

SampleOrder The order in which the sample being predicted. Note that in SpectrumPredict, only one sample can be predicted at a time.

CalibrationOrder The order in which the calibration type (i.e., experiment) is created.

ConsituentOrder The order in which the constituent is established when the training data file is created.

The prediction results for each prediction item (i.e., each row displayed in the *Prediction Results* group box) is also displayed in *Prediction Results for Item* group box on the lower left of the SpectrumPredict interface for easy viewing, as shown below.



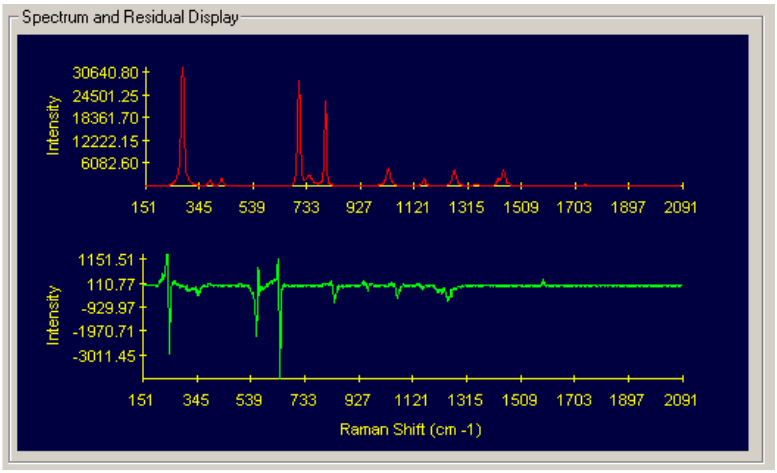
Prediction Message

The text message from a predict session is displayed in the *Prediction Message* group box of the SpectrumPredict interface:

Upon error conditions, an error message will be displayed, which is the same as that in the *Error* column of the *Prediction Results* listing.

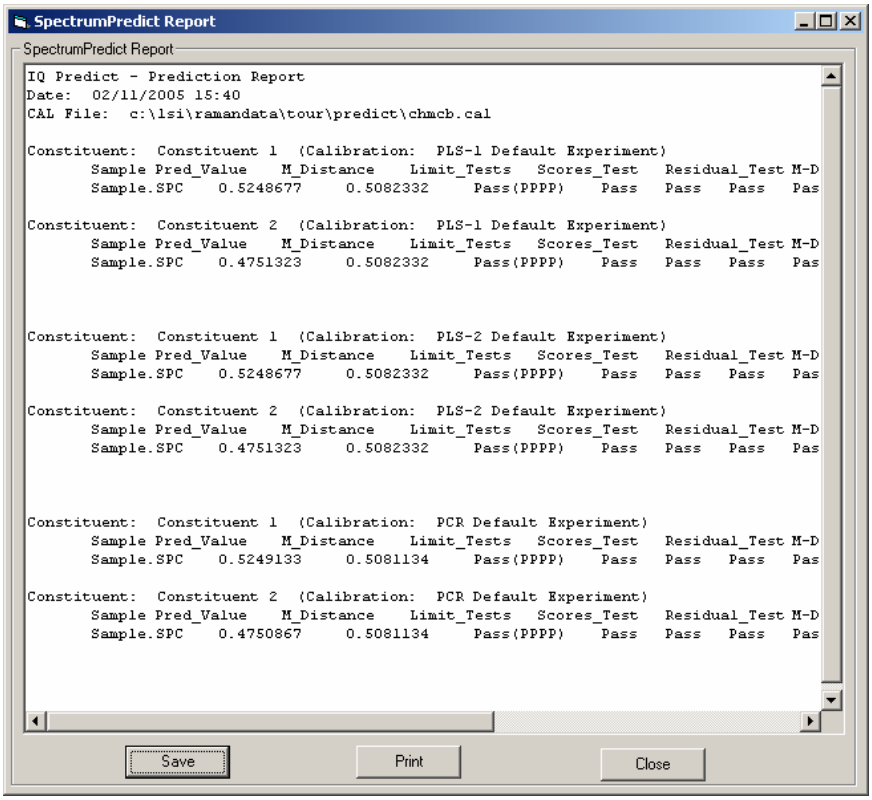
Spectrum and Residual Display

The sample spectrum and its spectral residual are displayed in the *Spectrum and Residual Display* group box of the SpectrumSearch interface. The residual spectrum is the difference between the sample spectrum and the “predicted spectrum”, a spectrum constructed from the prediction values.



Prediction Results Report

The prediction results can be viewed by pressing the *Report* button on the SpectrumPredict interface. The contents in the report is configured through the SpectrumPredict Configuration window.

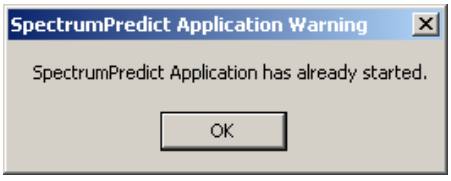


Press *Save* to save the prediction results as Rich Text File (RTF) or as plain text file (.txt). Press *Print* to print the report. Press *Close* to close the SpectrumPredict Report window.

SpectrumPredict Troubleshooting

SpectrumPredict Application Already Started

If the SpectrumPredict application is already started, and the user clicks on RamanSoft *Tools -> SpectrumPredict* or the SpectrumPredict icon  on the Function Toolbar, the following warning window will appear:



Click *Ok*, and locate the current SpectrumPredict application.

Two SpectrumPredict Application Interfaces

This might occur if the user has clicked *OK* on the SpectrumPredict Application Warning window (see “SpectrumPredict Application Has Already Started” in this section) and then proceeded to

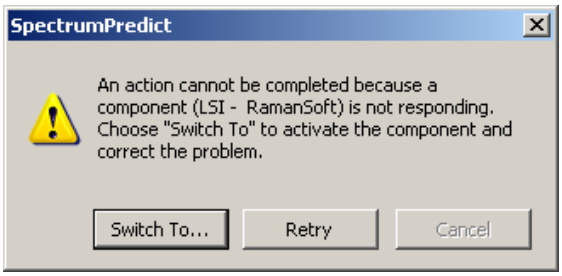
restart the SpectrumPredict application (see “Restarting the SpectrumPredict Application” in this section). In this case, the user can launch a second SpectrumPredict interface without being warned again. Close the newly started SpectrumPredict application.

No Action Occurred After Pressing *Predict*

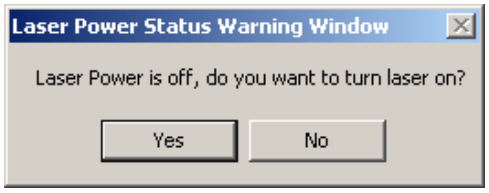
The laser might be off, as the system automatically turns the laser off if there is no sample acquisition for 30 minutes. Click on *System->Laser Setting->Laser On* to turn the laser on from the RamanSoft main menu (See section “RamanSoft Trouble Shooting” in Chapter 5 for more information).

SpectrumPredict Application Warning

If the following window appears after pressing *Predict* on the SpectrumPredict interface,



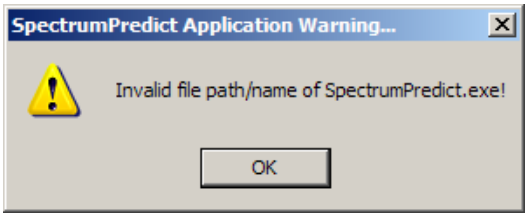
The laser might be off, as the system automatically turns the laser off if there is no acquisition for 30 minutes. Click on *Switch To*… button. The Laser Power Status Warning Window will appear.



Click *Yes* to turn the laser on. Wait for the laser power to stabilize (the progress bar of the Laser Power Stabilizing Progress Window reaches the end). Press *Predict* to continue.

Invalid File Path/Name of SpectrumPredict Executable

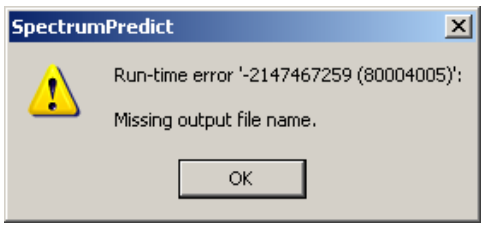
If the invalid file path/name of SpectrumPredict.exe error occurs as shown below:



The SpectrumPredict executable might have been relocated from its default location in C:\LSI\RamanSoft\exe\SpectrumPredict.exe. Proceed to “Restarting the SpectrumPredict Application” and browse to locate SpectrumPredict.exe from the right folder.

Missing Output File Name Error

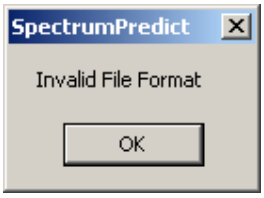
When passing an existing spectrum for prediction and there is no file name/path in the text field, the following error would appear:



Click *OK* to close the application, and proceed to the trouble shooting topic “Restarting the SpectrumPredict Application”. Enter the file path/name to save the unknown sample spectrum in SPC format from the SpectrumPredict interface, and press *Predict* to continue.

Invalid File Format Error

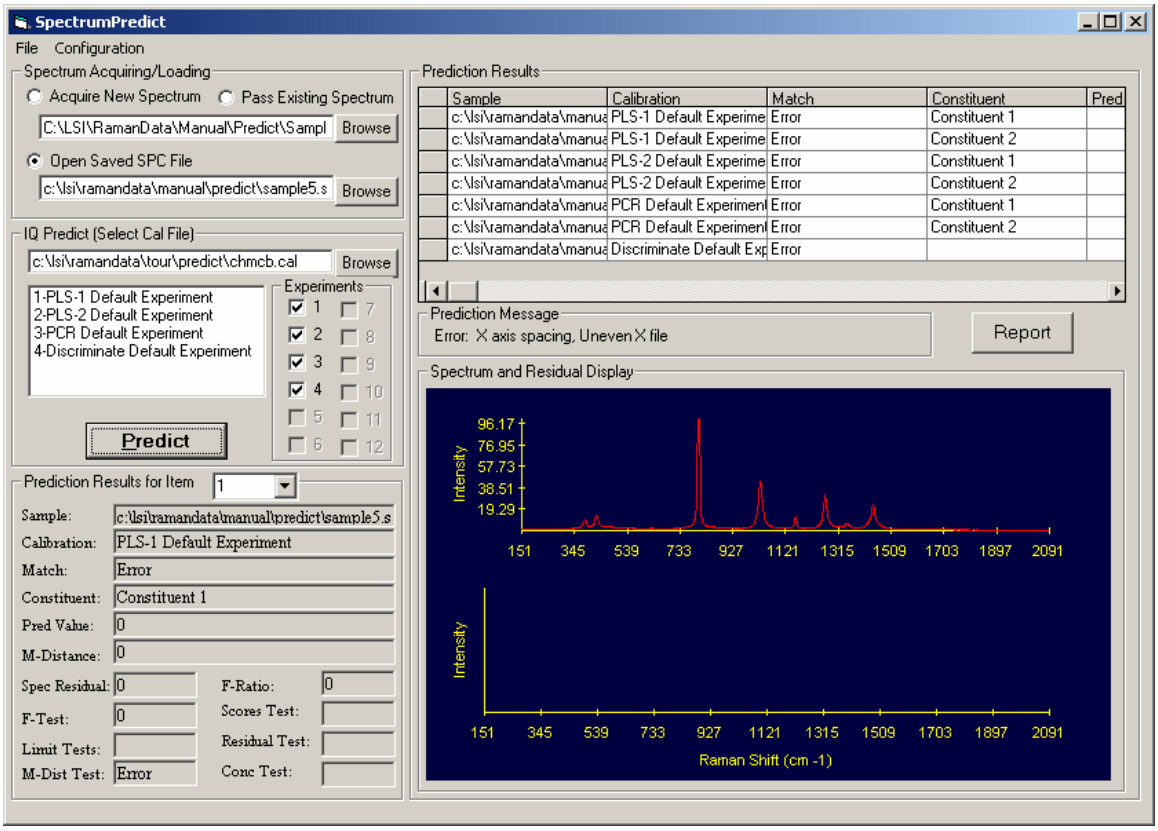
In saving prediction report through *File->Save*, if the user presses on *Cancel* button, the following error might occur:



Click *OK* to close the application, and proceed to the trouble shooting topic “Restarting the SpectrumPredict Application”.

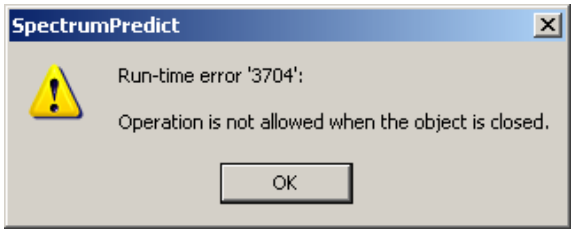
Prediction Error

If a prediction error occurs, the error message will appear in the *Prediction Message* group box. The *Match* column in the *Prediction Results* listing will display “Error”, and the residual spectrum will not be plotted as the sample spectrum is not predicted. The following figure shows a prediction error resulted from the fact that the training data set files and the sample spectrum differ in their x-axis scale.



Run-time Error

If run-time error occurs, such as



Press *OK*, and proceed to trouble shooting topic “Restarting the SpectumPredict Application”.

Unknown Error Conditions

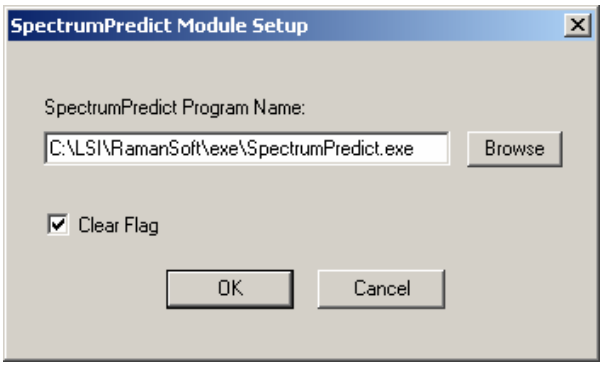
If the SpectrumPredict module crashes, or any other error conditions arise from this module, proceed with “Restarting the SpectrumPredict Application”.

Upon any other error conditions, exit both programs (exit SpectrumPredict first and then exit RamanSoft), and re-launch RamanSoft.

If neither of the above two procedures works, restart the computer.

Restarting the SpectrumPredict Application

Go to RamanSoft *System->SpectrumPredict*, and check the *Clear Flag* checkbox.



Click *OK*, and then restart the SpectrumPredict application by clicking on RamanSoft *Tools -> SpectrumPredict* or the SpectrumPredict icon on the Function Toolbar.

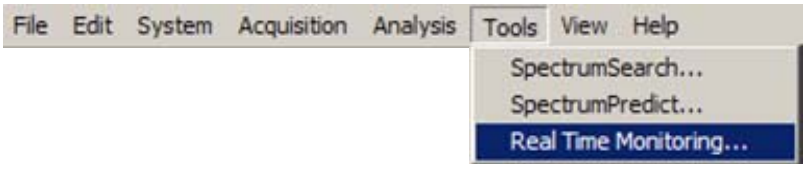
8 The Real Time Monitoring Module

Overview

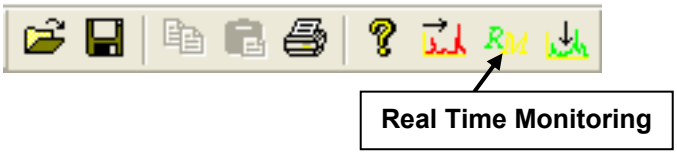
The Real Time Monitoring Module is designed to meet the need to monitor a process in real time. For example, in a chemical reaction process, the concentration of each reactant or the product may change as a function of the reaction time. When the Dimension Raman system is set to acquire the spectrum of the chemical reaction system, the characteristic peak intensities in the acquired Raman spectrum will change dynamically in response to the reaction process. The Real Time Monitoring module can monitor up to five peaks simultaneously either in peak intensity or in peak area as a function of time.

Launching the Real Time Monitoring Module

To start the Real Time Monitoring module, click on *Tools->Real Time Monitoring* from RamanSoft main menu.

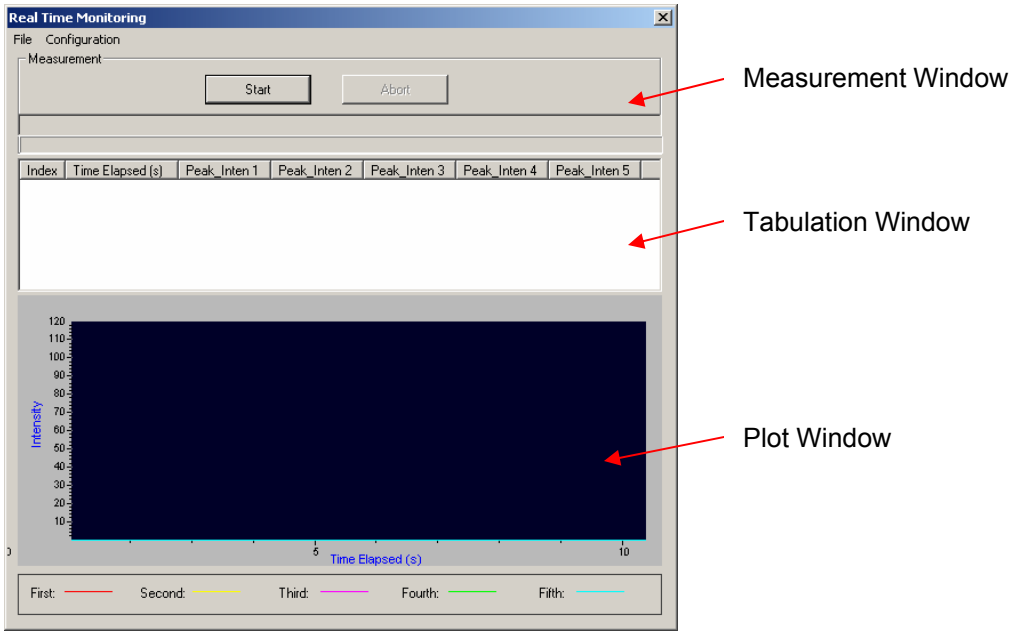


Alternatively, you can start the Real Time Monitoring module by clicking on  from the Function Toolbar.



The Real Time Monitoring User Interface

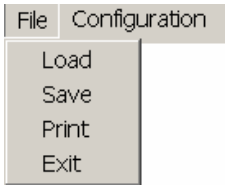
Upon module start-up, the Real Time Monitoring user interface appears as follows. The interface consists of the main menu, the Measurement Window, the Tabulation Window, and the Plot Window.



The main menu consists of the *File* and *Configuration* menu items.

The File Menu

The *File* menu contains *Load*, *Save*, *Print*, and *Exit* submenu items.



Load

This allows for loading the previously saved real time monitoring results and displaying them on the Real Time Monitoring interface.

Click *File->Load*, browse to the directory where the final Real Time Monitoring results are saved (Text files), choose a filename in the file Open window. Click *Open*. The previously saved results will be loaded and listed in the Tabulation Window and displayed in the Plot Window.

Save

This allows for saving the real time monitoring results into a text file.

After a Real Time Monitoring session has completed and the monitoring results have been displayed on the Real Time Monitoring interface, click *File->Save*. Type in a file name, and click the *Save* button. The results are saved in a text file.

The saved results can later be loaded into the Real Time Monitoring interface through *File -> Load*.

Print

This allows for printing the real time monitoring results onto the default printer that your computer connects to.

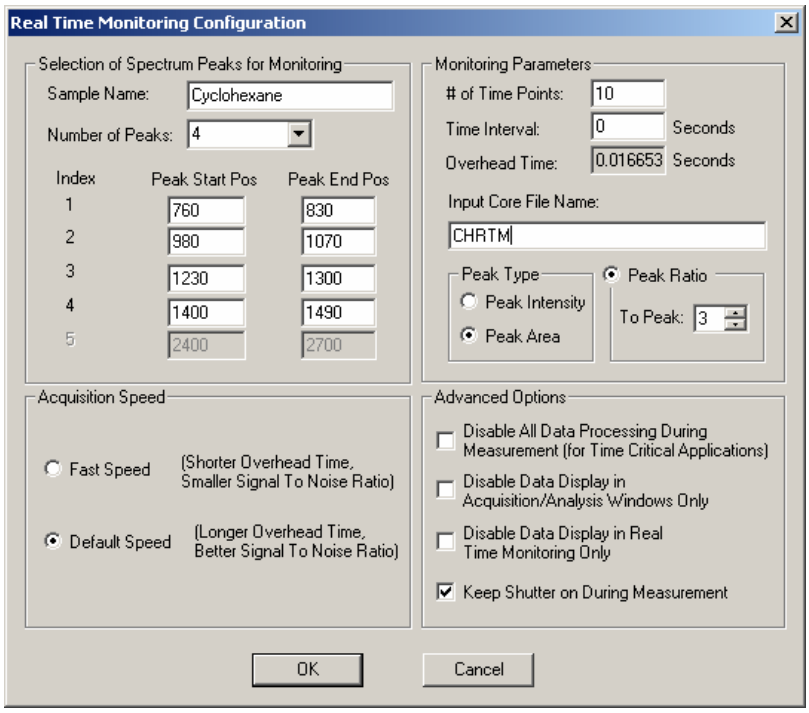
Exit

This exits the Real Time Monitoring module.

The Configuration Menu

The *Configuration* menu contains only one menu item, the *Configuration Setup*.

Click on *Configuration->Configuration Setup* to bring up the Real Time Monitoring Configuration window.



Selection of Spectrum Peaks for Monitoring

Enter the *Sample Name* for the sample to be monitored, and specify the *Number of Peaks* to be monitored. As many as 5 peaks can be monitored at the same time. The selection of the *Number of Peaks* will dynamically enable or disable the edit boxes for *Peak Start Pos* and *Peak End Pos*. Enter the peak start and peak end position values for the peaks of interest. Acquire a spectrum of the sample of interest and display it on the RamanSoft Acquisition Window or Analysis Window and use the cursor to locate the peak start and peak end positions. Click *OK* each time after entering the numbers before going back to RamanSoft Acquisition Window or Analysis Window to identify other peaks so that the entered information is saved by the program.

Monitoring Parameters

Enter the number of time points to monitor and the time delay between consecutive time points in the *# of Time Points* and *Time Interval* edit boxes, respectively. The maximum number of time points is 1024.

Enter the core filename in the *Input Core File Name* text field. For processes that are not time-critical, RamanSoft appends a sequential integer number to the core file name to form a filename for the spectrum at each time point and save it into the specified data folder (specified through RamanSoft main menu *Acquisition->Setup*). For time-critical processes (i.e., when *Disable All Data Processing During Measurement* is checked at the *Advanced Options* group box), only a multifile is saved, using the core file name appended with the total number of time points as the filename.

Select either the absolute peak intensity or the peak area for monitoring, or the peak ratio calculated either by peak intensity or peak area. The *Peak Intensity* is the maximum peak intensity between the peak start position and the peak end position. The *Peak Area* is calculated by summing all the intensity values of the spectrum points between the peak start position and the peak end position. The *Peak Ratio* is the ratio of the peak intensity or peak area values to that of the peak specified in *To Peak* selection box.

Acquisition Speed

This allows the user to choose fast or standard (or, default) CCD digitization speed, each of which has different overhead times (data readout time). *The Fast Speed* gives shorter overhead time but lower signal-to-noise ratio. It is recommended for acquisition times less than 50 milliseconds. *Default Speed* gives longer overhead time but better signal-to-noise ratio. The overhead time from CCD readout delay is displayed in the *Overhead Time* textbox in the *Monitoring Parameter* group box.

Note that the *Acquisition Speed* option shown here is the same as that in the CCD Camera Parameter Setup window accessed from RamanSoft main menu *System->CCD Configuration*. But for Real Time Monitoring applications, this speed needs to be configured here to be effective.

Advanced Options

Disable All Data Processing During Measurement. Check this option to disable all data processing including file saving and data displaying during real time monitoring in order to achieve minimum overhead time for time-critical processes. When this option is chosen, no data display is available and only the Real Time Monitoring progress bar is active. The data processing, file saving, and data displaying are performed at the end of the real time monitoring process.

Note that when this option is chosen, only a multifile is saved at the end of the real time monitoring process. No individual files (i.e., single files) are saved for each time point.

Disable Data Display in Acquisition/Analysis Windows Only. Check this option to disable data display in the RamanSoft Acquisition Window and Analysis Window only. The Real Time Monitoring interface remains active, i.e., it displays the tabulated intensity or area data in the Tabulation Window and the plotted data in the Plot Window. This option allows for a lower overhead time for a real time monitoring process.

Disable Data Display in Real Time Monitoring Only. Check this option to disable data display in the Real Time Monitoring interface only. Disable this data display to give less overhead time.

Keep Shutter on During Measurement. Check this option to keep shutter on all the time during the real time monitoring process. This is recommended for process monitoring with small time delays (i.e., *Time Interval*) between measurement points (e.g., < 1 s). This would help to minimize the mechanical wearing of the shutter by keeping it from frequent opening and closing.

Performing a Real Time Monitoring Measurement

Proceed with the following steps to perform a real time monitoring measurement:

1. Set up real time monitoring configurations in the Real Time Monitoring Configuration window.

2. Set up acquisition parameters from RamanSoft main menu:

    a. Set up CCD integration time and other CCD camera parameters through *System -> CCD Configuration*.

    b. Set up file *Path* through *Acquisition->Setup*. This is where all the files from Real Time Monitoring as well as from RamanSoft are saved. Input sample name, sample info, and file prefix.

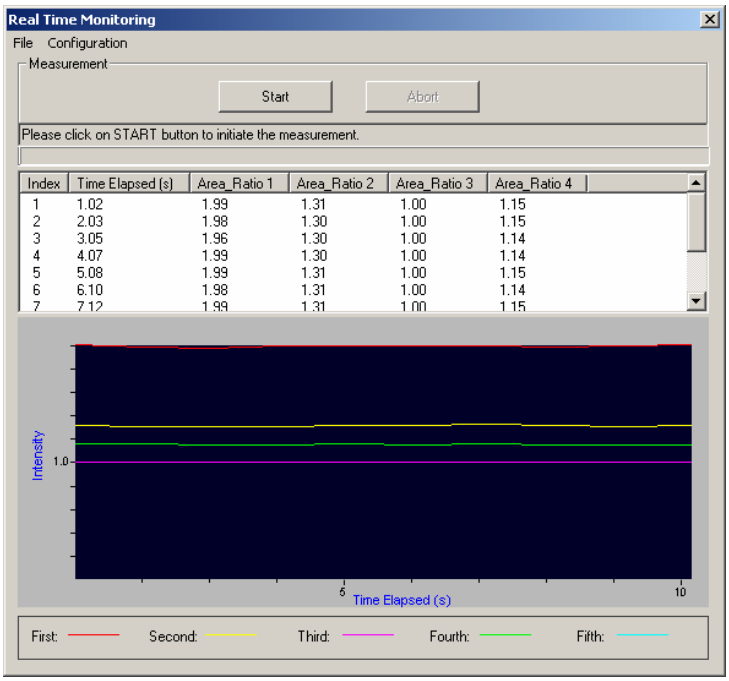
    c. Set up data processing methods through *System->Automation->Setup*.

    d. Configure data processing algorithms through *Analysis->Algorithms Setup* for the chosen methods in c. Remember to click *Apply* button on the Algorithm Setup page after an algorithm is configured.

    Note: If no data processing methods are configured in System Automation Setup window, the user needs to make sure that *System->Automation->On* is checked so that the individual files can be saved for each measurement point during the Real Time Monitoring process.

3. Put the sample to be monitored in the sample cell.

4. Press *Start* button on the Real Time Monitoring interface. The progress bar displays the measurement progress. At each measurement point, the program acquires one spectrum and display it in the Acquisition Window, as well as in the Analysis Window if *System->Automation->On* is checked. The calculated peak intensities or peak areas or peak ratios are tabulated in the Tabulation Window and displayed in the lower Plot Window of the Real Time Monitoring interface (Note: the data display depends on the configuration of the *Advanced Options* in the Real Time Monitoring Configuration window).



The vertical axis of the plot is either the peak intensity or the peak area depending on the selection of parameter to monitor. The scale of the vertical axis will be dynamically adjusted at each measurement point so that the vertical display range covers all the data points. The horizontal axis of the plot is “Time Elapsed” (in seconds). The range of the horizontal scale is calculated and updated for each real time monitoring configuration.

The “Time Elapsed” quantity is defined as following:

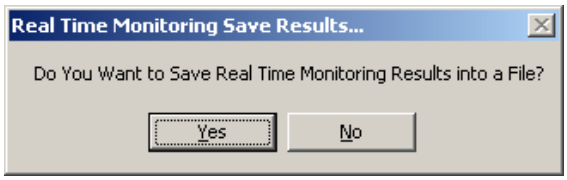
$T_{Elapsed}$ (i) = CCD_Integration_Time * (i + 1) + Time_Interval * (i) + Overhead_Time *(i+1)

Where i = 0, 1, 2, ….n - 1 and n is the total number of time points. For the first time point, i = 0.

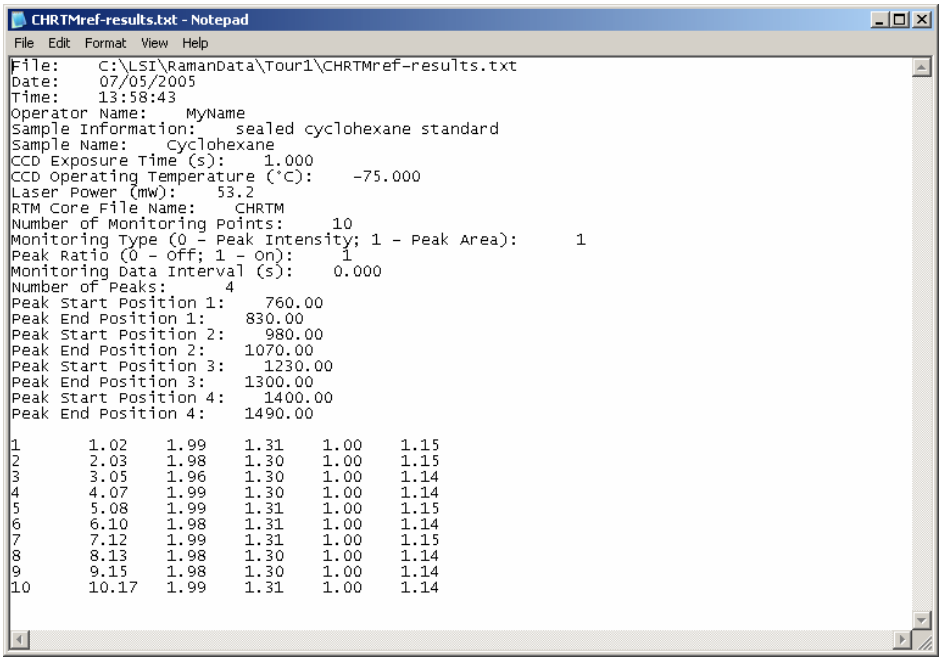
5. During the real time monitoring measurement, you are able to abort the measurement any time by clicking on the *Abort* button (Note: Please be patient after clicking the *Abort* button. The program needs a delay time to respond to the abort action. This is especially the case if the CCD integration time at each measurement point is long (e.g., > 3 seconds), or the time interval between each data point is large (e.g., > 10 seconds)).

6. When the monitoring process is completed (the progress bar reaches the end), RamanSoft automatically takes one additional acquisition and saves the spectrum in the location and with the filename as configured in *Acquisition->Setup*. This action resets RamanSoft configurations to the values prior to the Real Time Monitoring application so that the system is ready for next regular RamanSoft acquisition.

7. The user is prompted to save the results into a text file.

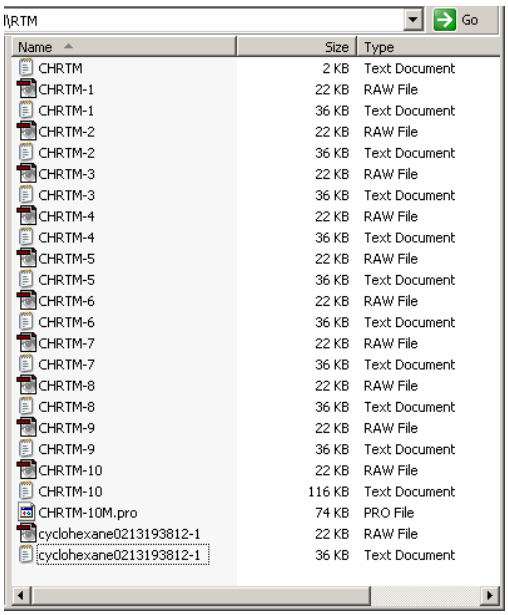


The user can save the results by pressing *Yes*, and input a filename. The user can also choose *File->Save* to save the results into a text file. Either way, the saved file contains the system parameters as well as configuration settings in the file header, and columns of data entries: the time point index, the time elapsed (in seconds), and peak intensity or peak area for each monitored peak.



File Operations

There are several sets of files generated and automatically saved for a Real Time Monitoring application; the exact number of sets depends on the configuration settings. The following figure shows the files generated in a none time critical real time monitoring process in which no data processing methods are selected (so that there are no *.pro files saved for each time point).

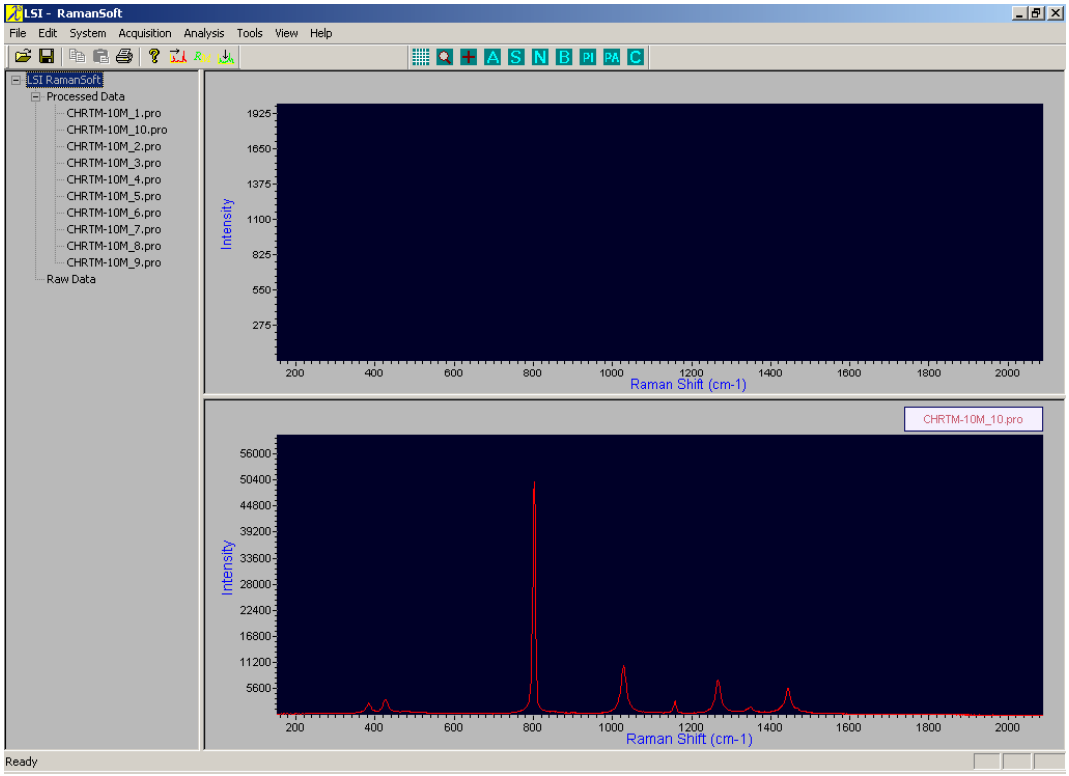


1. Individual files (also termed “single files”) for each measurement point. These files are named using the *Core File Name* appended with an index number for each time point, and they are regular RamanSoft files (i.e., *.raw files, *.pro files if any data processing methods are configured through *System->Automation*, *.txt files, and *.spc files if *GRAMS Format* option is checked in *File->Preferences*). In the above figure, files CHRTM-1…CHRTM-10 are such files.

   Note that for time critical process (i.e., the *Disable All Data Processing During Measurement* option is checked in the Real Time Monitoring Configuration window), these single files are NOT saved.

2. A multifile for the RTM application. This file (in LSI “.pro” file format) contains all the spectral data at each measurement point. In the above figure, CHRTM-10M.pro is such a file. The “M” in CHRTM-10M.pro indicates that this is a multifile; this file contains all the spectral data for 10 measurement points.

   To open a multifile, go to RamanSoft *File->Open*, browse to the directory where the multifile is located, choose the filename, and click *Open*. The multifile is automatically displayed as single files, each of which contains the spectral data at one measurement point. These files are listed in the File View Window, and the last one is displayed in the Analysis Window. Note that the program appends a sequential integer number to the multifile filename to form the filename for each temporary single file.



Double click on a filename in the File View Window, the spectrum will then be displayed in the Analysis Window. The user can save this file by clicking on *File->Save As->Analysis Window*.

Note that these single files are temporary files stored in C:\RamanData\Temp. When RamanSoft is exited, the entire Temp folder is deleted.

3. Regular RamanSoft files (they are also single files) acquired at the end of the real time monitoring process. These files take the filenames using the *File Prefix* in the Acquisition Setup window appended by a time string (e.g., cyclohexane0213193812-1.raw, cyclohexane0213193812-1.txt).

4. The Real Time Monitoring result file. This is a text file that the user saved at the end of the real time monitoring process and can later be loaded into the Real Time Monitoring interface to be viewed (e.g., CHRTM.txt.

9 Virtual RamanSoft

Overview

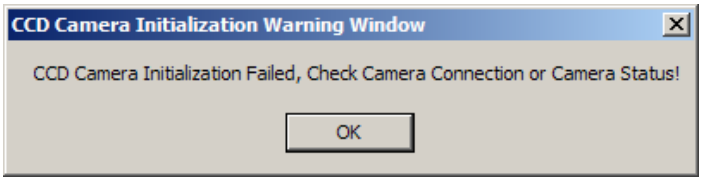
This chapter briefly describes how to operate RamanSoft without turning on the Dimension Raman System. This can be used, for example, performing offline data analysis.

Using Virtual RamanSoft

1. From Windows® *start* menu, go to *All Programs* and choose *LSI->RamanSoft*, or double click the RamanSoft shortcut icon on the desktop.

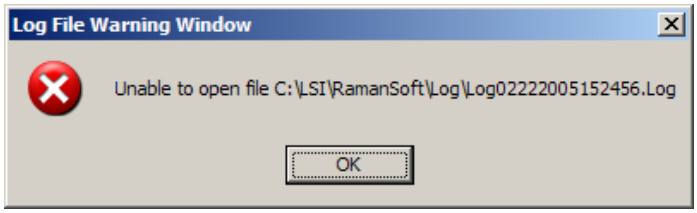
2. Choose the configuration file, and click *Open*.

3. The CCD camera initialization would fail, since the Dimension Raman System is not turned on for RamanSoft to communication with the camera.



4. Click on *OK* to accept the hardware status.

5. RamanSoft would start, and a warning would appear to warn the user that a log file will not be able to be created.



6. Click *OK* to accept the fact that no log file will be created for the offline RamanSoft session.

7. Proceed to use RamanSoft to perform data analysis. Note that in this offline mode, no data acquisition action can be performed.

8. Exit RamanSoft by choosing *File->Exit*, the user can choose to save the configuration file before exiting.

Appendix I System Specifications

Raman Systems	Dimension-P1™		Dimension-P2™	
	SR Model	HR Model	SR Model	HR Model
Spectrometer				
Optics	85 mm, f/1.8 lens-based, Czerny-Turner spectrograph			
Nominal Resolution	2.5 cm^{-1} /pixel	1.5 cm^{-1}/pixel	3 cm^{-1}/pixel	2 cm^{-1}/pixel
Coverage	150 - 3300 cm^{-1}	150 - 2075 cm^{-1}	150 - 3000 cm^{-1}	150 - 1950 cm^{-1}
Sensitivity(@100 mW w/785 nm laser)	>10,000 counts at 992 cm^{-1} of Na_2SO_4 @ lowest of 3 available gain settings		>5,000 counts at 992 cm^{-1} of Na_2SO_4	
Detector				
CCD	1340 × 100 pixels, 20 µm × 20 µm		1024 × 124 pixels, 24 µm × 24 µm	
TE Cooling	< -70°C		< -15°C	
Digitization Rate (16 bits)	100 kHz, 2 MHz		100 kHz	
Excitation Lasers				
Laser	785 nm (other wavelengths at 632.8 nm and 532 nm available)			
Laser Power	Up to 700 mW (0 - 350 mW to the sample) - Stability ±1%			
Sampling				
Probe	LSI Vector Raman Probe™, 1 meter fiber cable standard (other lengths available)			
Sample Cells	4-position internal sample cell and multi-purpose External Sampling Module		Multi-purpose External Sampling Module	
Software				
RamanSoft™	Control of laser power, CCD settings, data acquisition, processing & analysis including quantification, Real-Time Monitoring, SpectrumSearch™ , SpectrumPredict™			
Dimensions				
L × W × H	52 cm × 39 cm × 20 cm		39 cm × 25 cm × 15 cm	
Weight	18.5 kg		10 kg	
Options				
Microscope	Nikon and Olympus Microscopes, other brands available			
Microscope Adapter	Raman Microscope Adapter to microscopes			
Calibration Kit	Sources and tools for wavelength and instrument response calibration. Sealed sample for laser wavelength calibration			
Add'l Software	GRAMS/AI™ 7 with PLSplus/IQ™ add-on, or IQ Predict™; Spectral ID®			

Appendix II Mercury Lamp Peaks for Wavelength Calibration

Figure IIA. *Mercury Lamp Peaks Identified by a* ***Dimension-P1 HR*** *Raman System*.

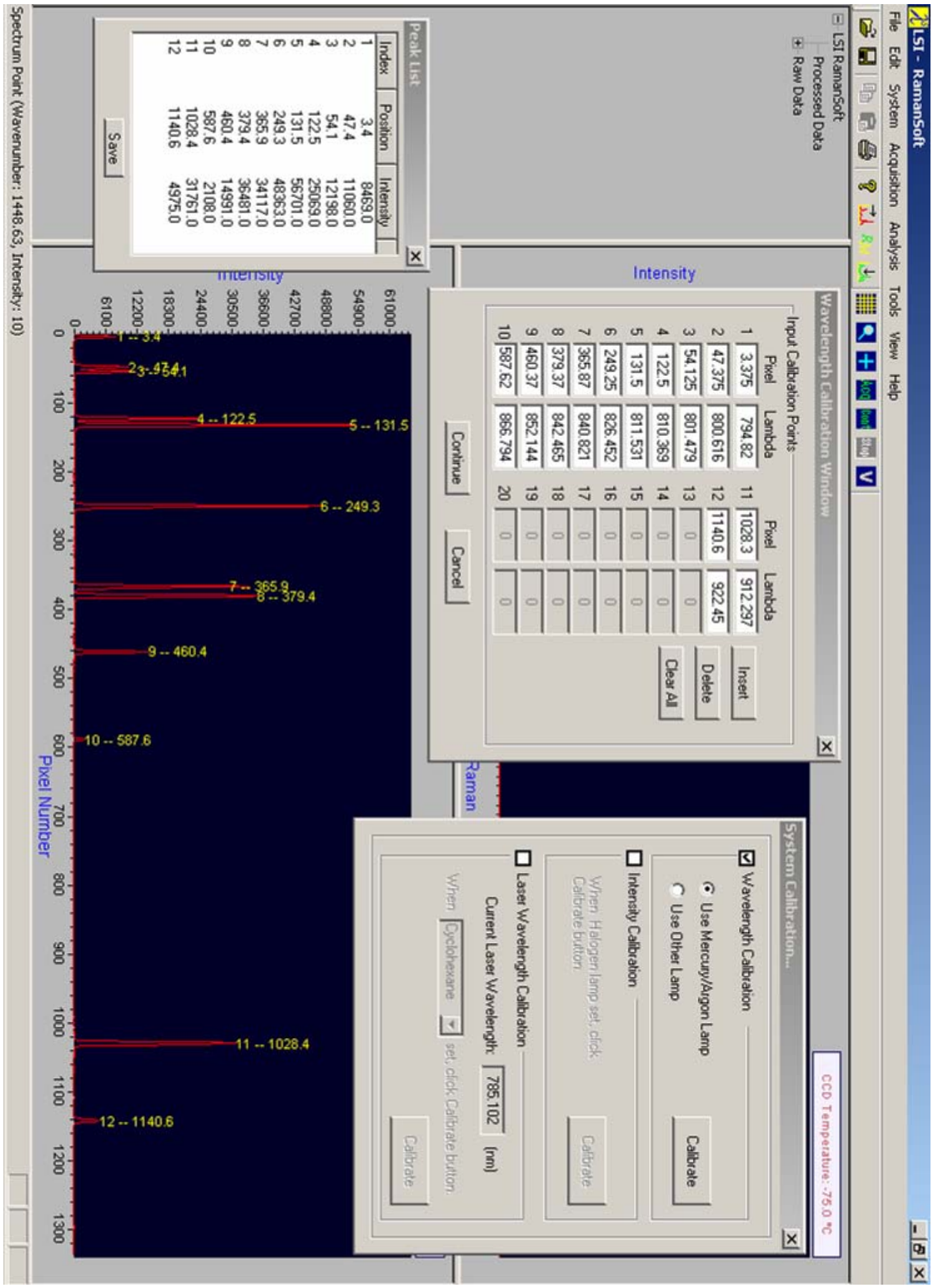


Table IIA. *The Standard Mercury/Argon Lamp Peaks for Auto Wavelength Calibration for* ***Dimension-P1 HR*** *Raman System.*

Peak Index	Pixel Number	Wavelength (nm)
1	3	794.82
2	47	800.616
3	54	801.479
4	122	810.369
5	131	811.531
6	249	826.452
7	366	840.821
8	379	842.465
9	460	852.144
10	588	866.794
11	1028	912.297
12	1141	922.45

This table lists the 12 mercury/argon lamp peaks used for the auto wavelength calibration on a Dimension-P1 HR Raman System. The Peak Index column lists the peak number, the Pixel Number column lists the approximate pixel positions of the peaks, and the Wavelength column lists the standard wavelength (in nm) for each corresponding peak.

Figure IIB. *Mercury Lamp Peaks Identified by a* ***Dimension-P1 SR*** *Raman System.*

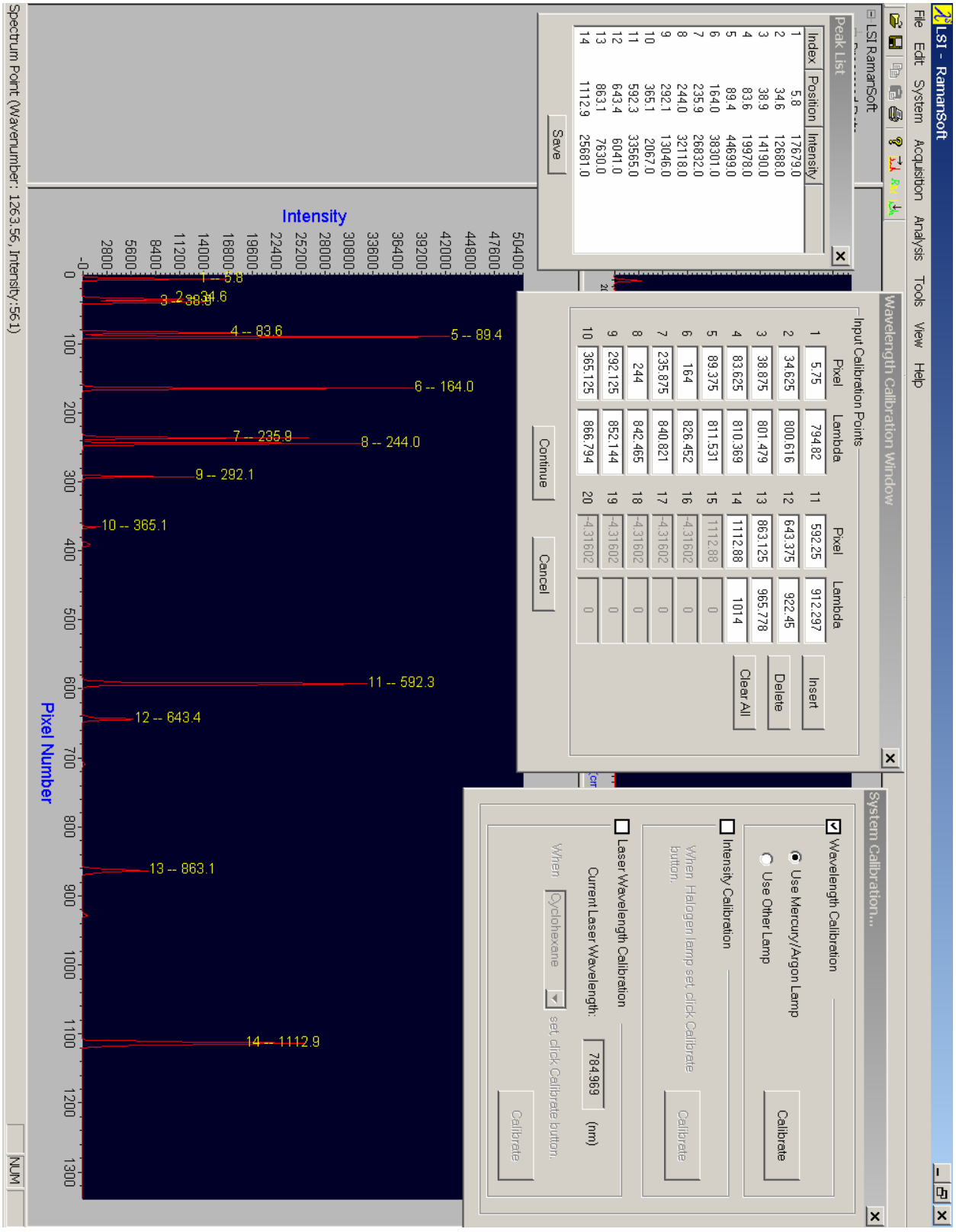


Table IIB. *The Standard Mercury/Argon Lamp Peaks for Auto Wavelength Calibration for* ***Dimension-P1 SR*** *Raman System.*

Peak Index	Pixel Number	Wavelength (nm)
1	6	794.82
2	35	800.616
3	39	801.479
4	84	810.369
5	89	811.531
6	164	826.452
7	236	840.821
8	244	842.465
9	292	852.144
10	365	866.794
11	592	912.297
12	643	922.45
13	863	965.778
14	1113	1014

This table lists the 14 mercury/argon lamp peaks used for the auto wavelength calibration on a Dimension-P1 SR Raman System. The Peak Index column lists the peak number, the Pixel Number column lists the approximate pixel positions of the peaks, and the Wavelength column lists the standard wavelength (in nm) for each corresponding peak.

Appendix III The Maximum Peak of Cyclohexane for Laser Wavelength Calibration

Figure IIIA. *Maximum Cyclohexane Peak Identified by a* ***Dimension-P1 HR*** *Raman System.*

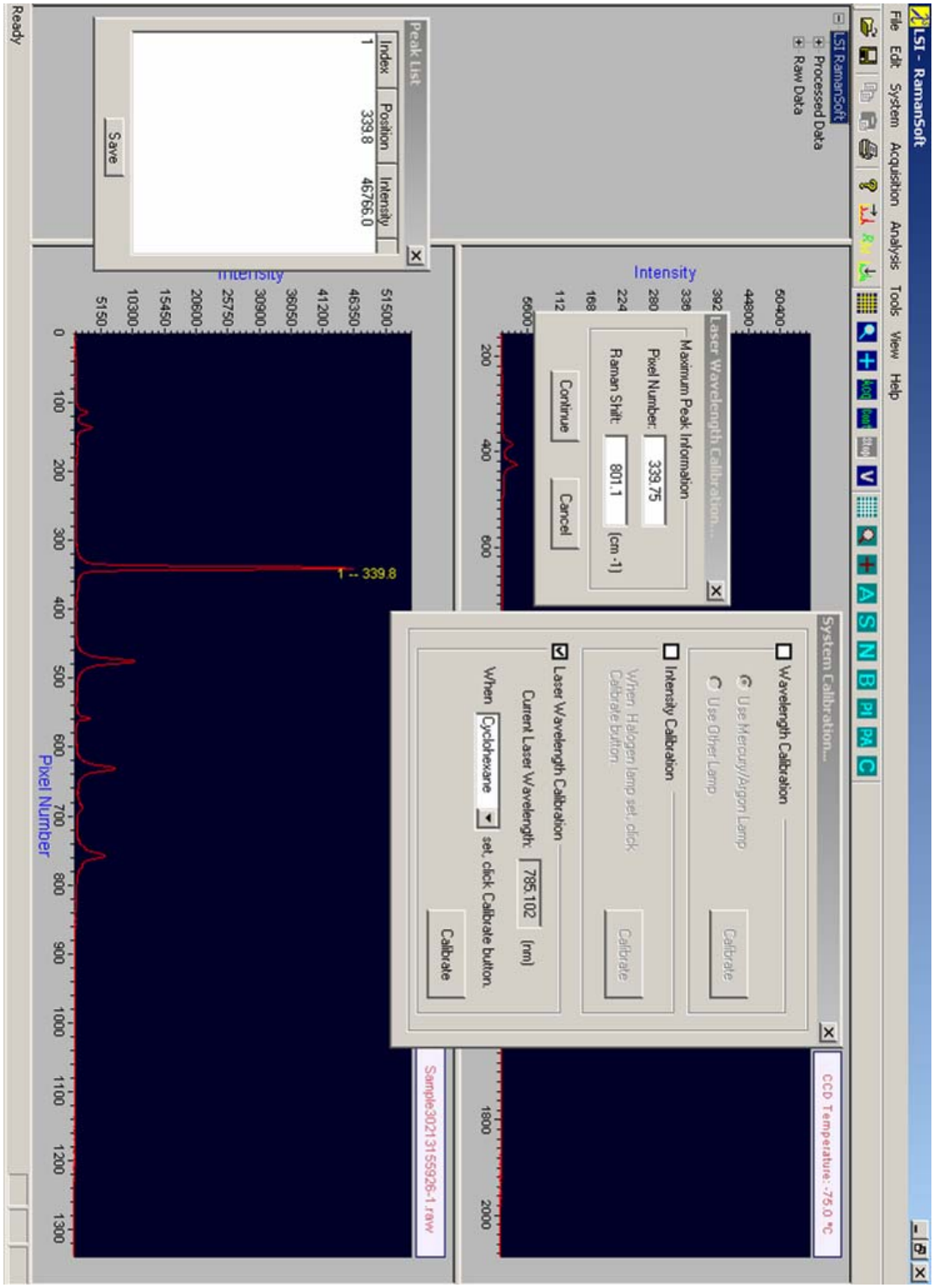
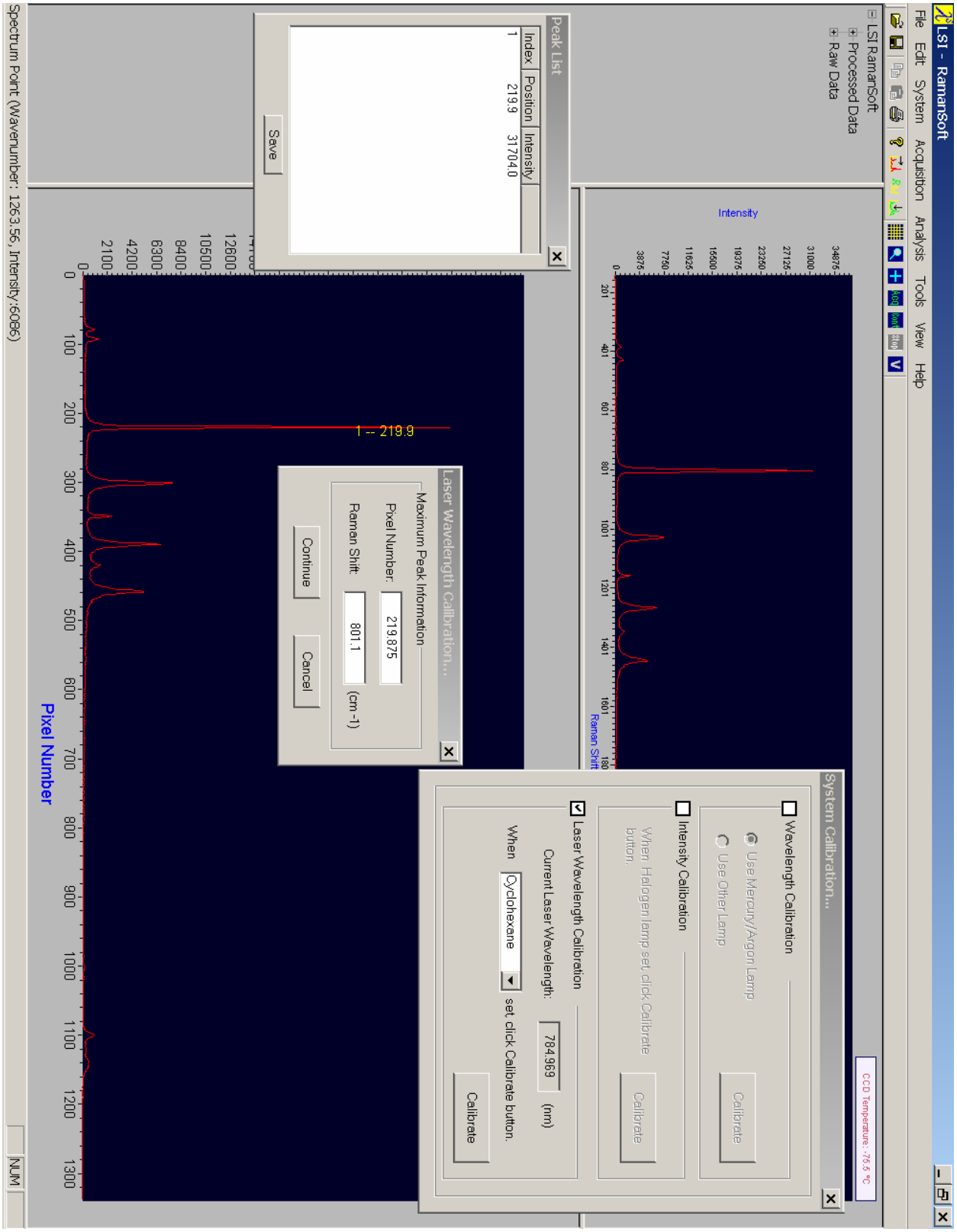


***Figure IIIA**. Maximum Cyclohexane Peak Identified by a **Dimension-P1 HR** Raman System.*



Warranty & Service

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User Manual and Troubleshooting

LSI has endeavored to make the User Manual comprehensive and complete with respect to the Dimension Raman unit (Dimension-P1™, Dimension-P2™) and accessory hardware assembly and the operation of RamanSoft™ software. You should read this manual thoroughly before operating this product.

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   - had serial numbers removed or altered in any way or which are illegible;
   - been serviced by unauthorized personnel;
   - been damaged as the result of environmental conditions, liquid spills, fire, flood, radiation or other "acts of God" or conditions beyond the control of Lambda Solutions.

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