

***Renishaw inVia Raman Microscope
Training Notebook***

NanoTech User Facility (NTUF)

Center for Nanotechnology

University of Washington

December 2007

Starting the System

☞ The main system unit is ON at all times.

514 nm

☞ Power on the desired laser(s):

- 514 nm laser: switch the key on the small control box to emission and slowly increase the power to the indicated mark (15 mW).
- 785 nm laser: turn key on laser (black box behind microscope) until status lights turn green.



☞ Power on the controller for the Prior xyz stage (under monitor) .

785 nm



☞ Switch on the light source (orange rocker) on the LEFT side of the Leica microscope base for brightfield (BF) viewing and adjust intensity with dial under the base. Use the rocker switch on the RIGHT to select either reflective (up) or transmission lamp (down).

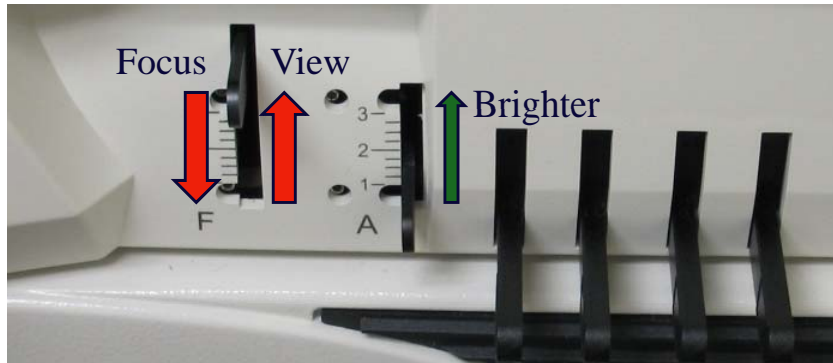
☞ Start the Renishaw WiRE 2.0 software.



Laser safety: Class 3b laser lines are present during signal acquisition and opening of the Renishaw access door. Do not look directly at the beam. Do not interfere or modify the beam path.

Calibration on Si(100)

1. Lower the sample stage with the coarse adjustment and load the Si(100) target with arrows pointing away for consistency. Select desired objective (10x for viewing, 50x for spectra).
2. Switch the top and bottom turrets to brightfield (2/1) and slowly focus on the Si(100) surface. Closing the field diaphragm is recommended for reflective samples.
3. Activate Raman collection channel by switching the turrets to (1/2) as listed in Table below.

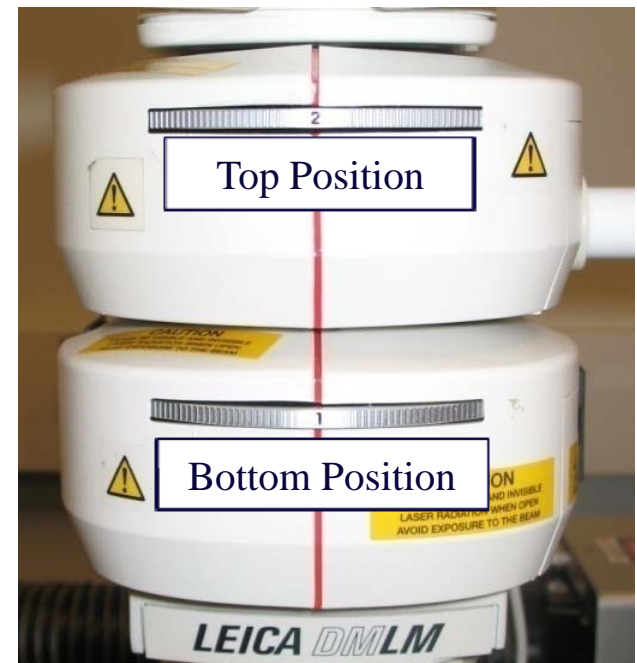


To focus on the surface, close the field diaphragm (F) and gently raise the sample until a octagon pattern is visible. Open field diaphragm for viewing.

Eyepiece	B.F. 2/1*	D.F. 2/3**
Vis Spectra	B.F. 1/1	D.F. 1/3
Raman Spectra	1/2	

* The positions on the top and bottom turrets, respectively.

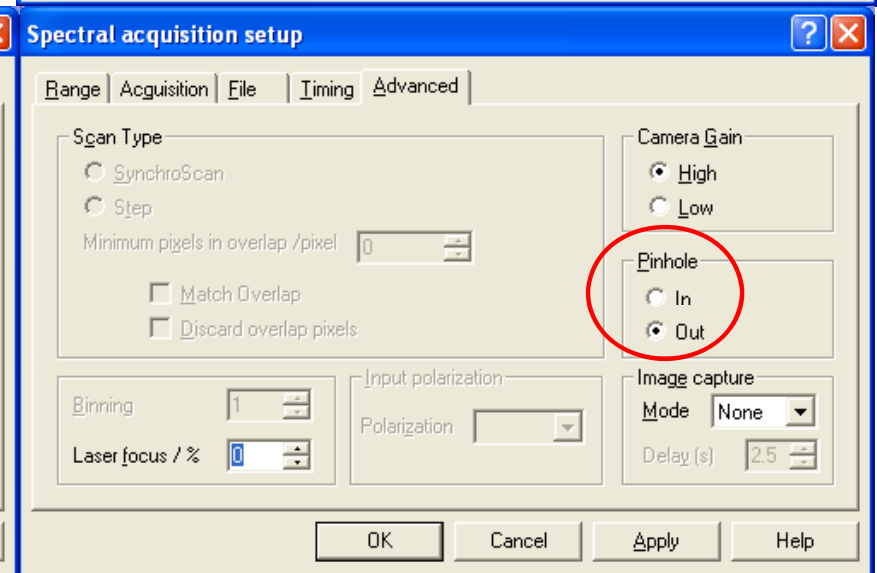
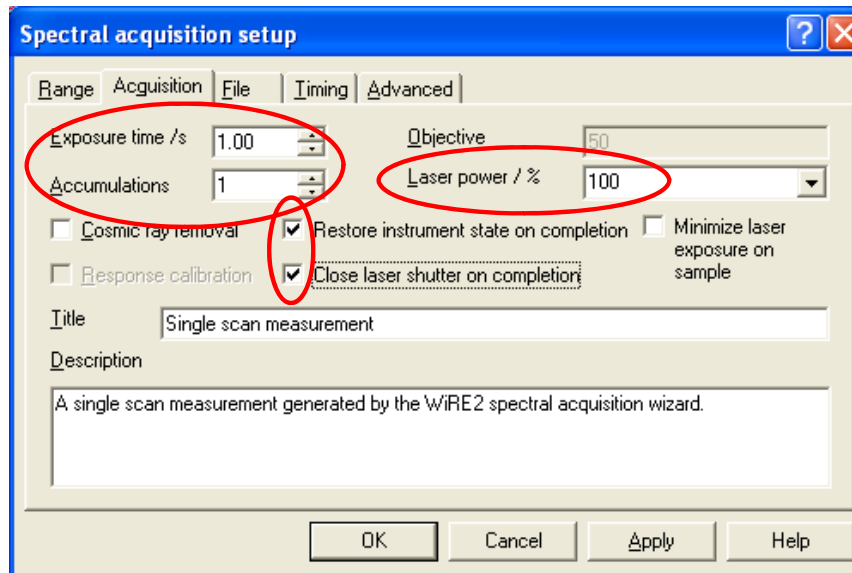
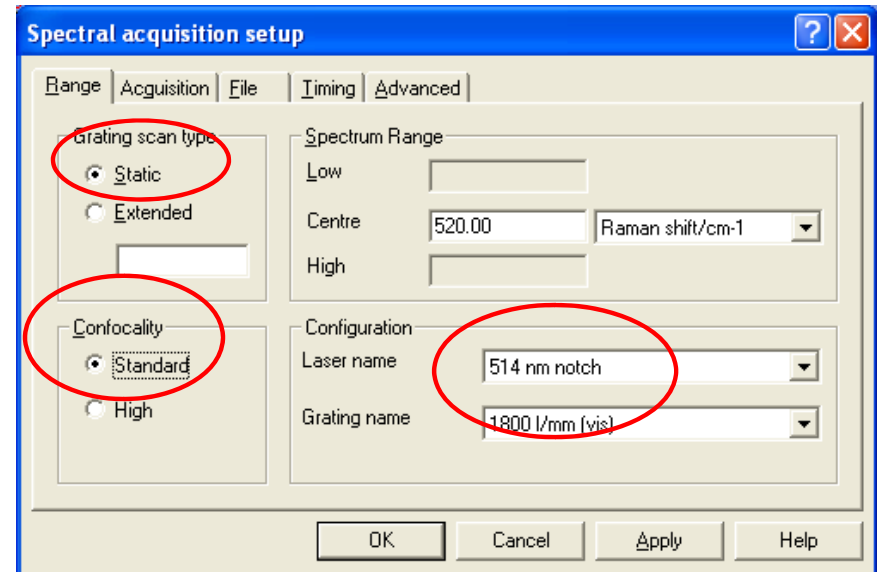
** 50x or 100x Dark Field objective only.



Calibration on Si(100)

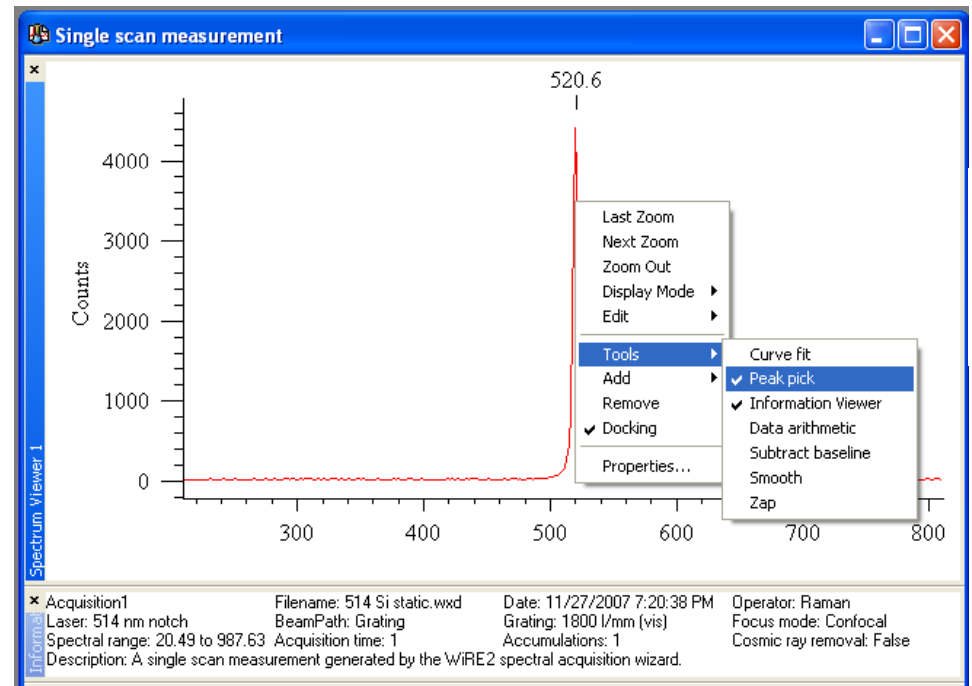
5. Open *Measurement>New>Spectral Acquisition* and use *Spectral acquisition setup* to define experimental parameters.

[Configuration for collection of Si peak at 520 cm^{-1} with 514 nm with standard confocality]



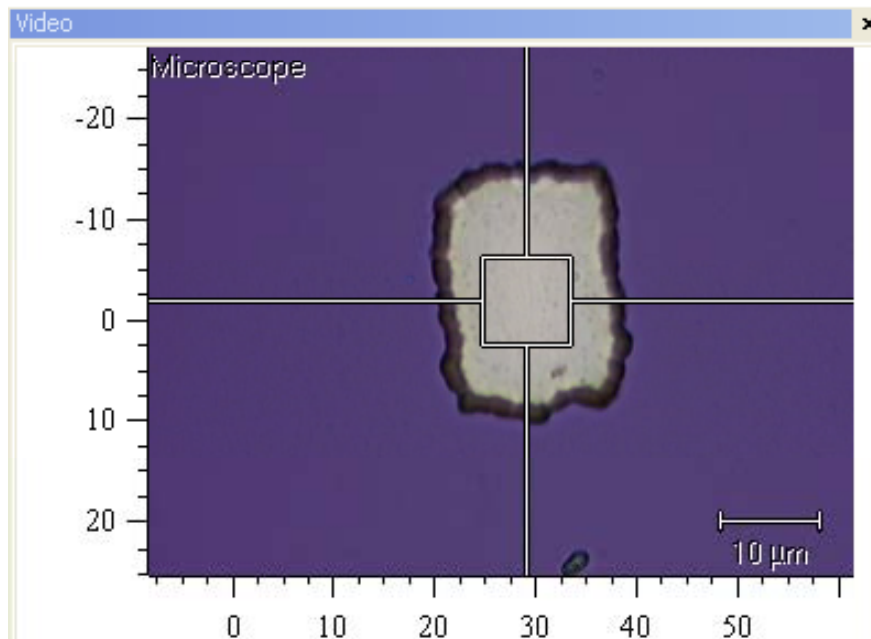
Calibration on Si (100)

6. Use *Measurement>Cycle* to collect static LIVE Raman spectra of Si(100) at 520 cm^{-1} and use focus knob next to the joystick to optimize signal. Click **Abort** to stop collection
7. Use *Measurement>Run* to collect static Raman spectra.
8. Zoom into the peak at 520 cm^{-1} . Right click to bring up options to check peak position. If it is not in the 520-521 cm^{-1} range, calibrate the system by clicking *Tools>Calibration>Quick Calibration*. Repeat steps 7-8.
9. Typical intensities of Si(100) at 514 nm and 785 nm excitation wavelengths are given in Appendix 1.

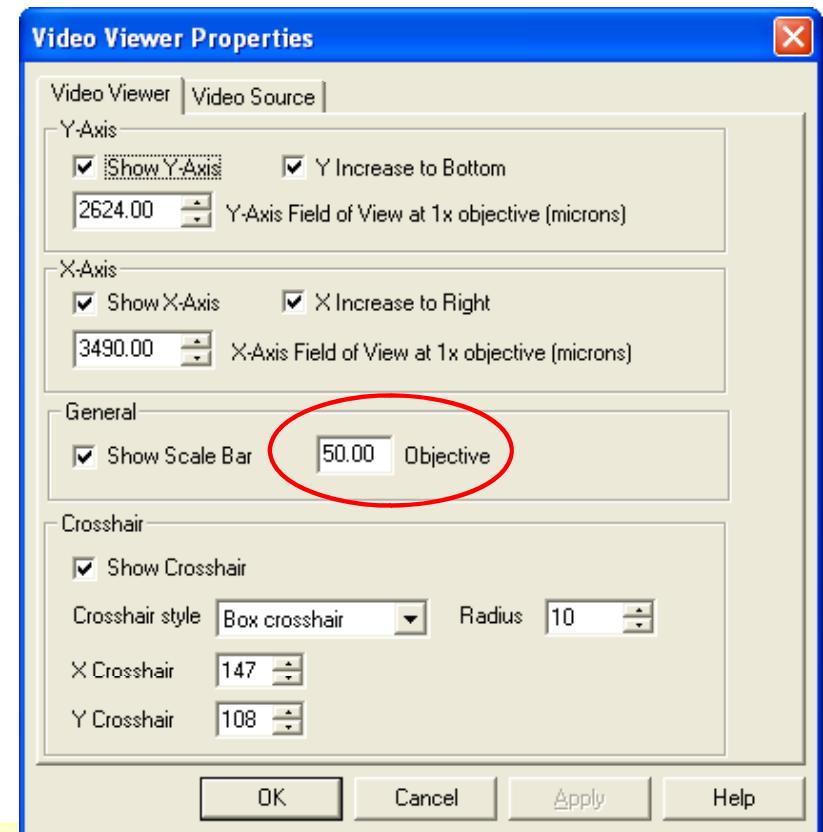


Collecting Spectra on Samples

1. Lower stage to place sample and select objective. Set magnification in *Video Viewer Properties* with right click on *Video window* to match current objective.
2. Use *Measurement>New>Spectral acquisition* to configure spectral measurement
3. Use for brightfield or darkfield viewing to locate target and focus. Move feature to the Region of Interest (ROI) in the cross hairs. Spectra will be collected where the laser impinges.
4. Use *Measurement>Run* or the *Run* icon to collect spectra.

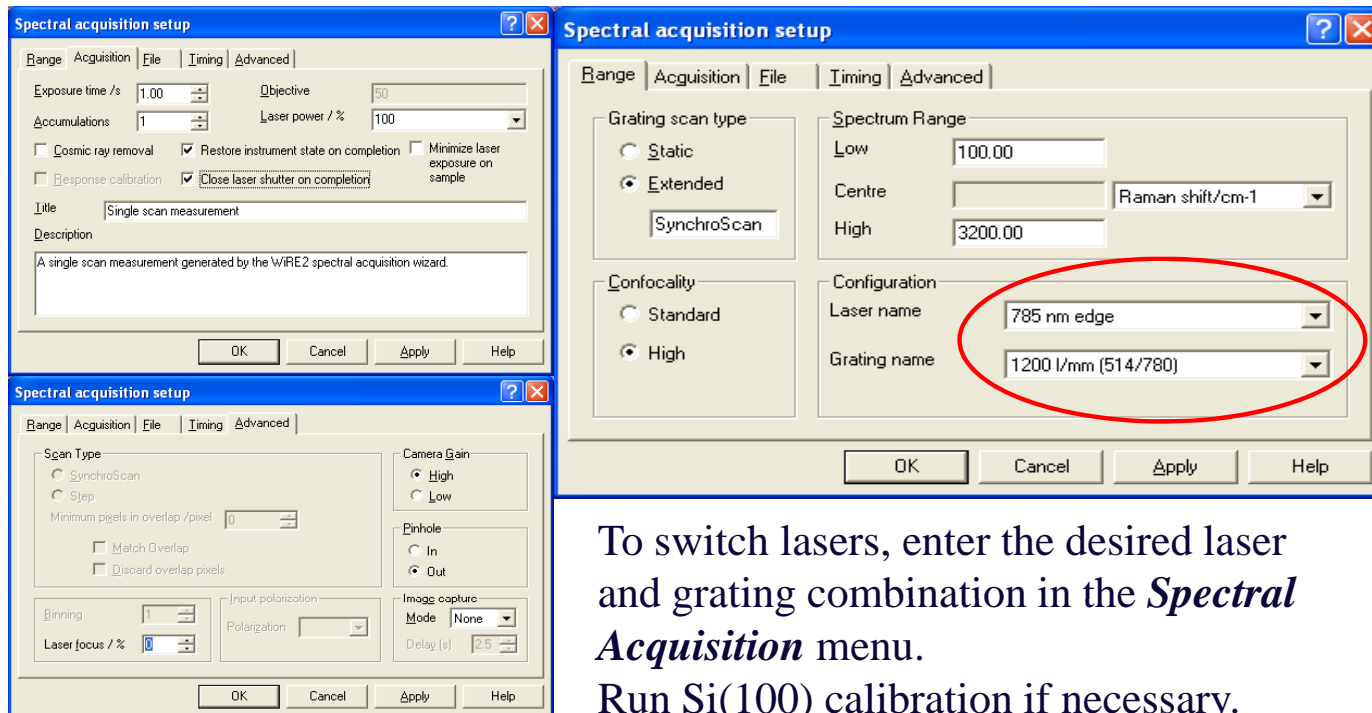


Do NOT change the position of cross hair



Switch Lasers

4. Use **Measurement>New>Spectral acquisition** to start a new spectral measurement:
 1. Laser excitation source and corresponding optics.
 2. Static vs. extended scans to capture the spectral region of interest
 3. Standard vs. high confocality mode for thin samples
 4. Exposure time and number of accumulations
 5. Pinhole in for spatial resolution with 785 nm laser
 6. Appropriate laser power to avoid sample damage
5. Use **Measurement>Run** or the **Run** button to collect spectra.

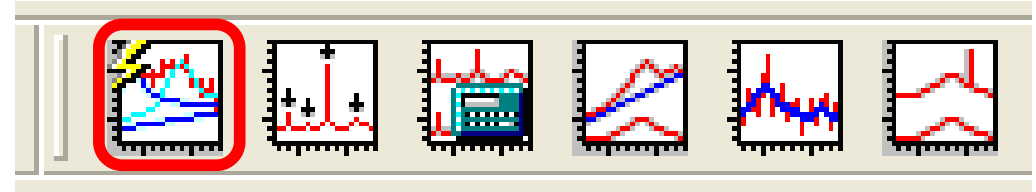


To switch lasers, enter the desired laser and grating combination in the **Spectral Acquisition** menu.

Run Si(100) calibration if necessary.

Curve Fit

Curve Fit Peak Pick Arithmetic Baseline Smooth Zap

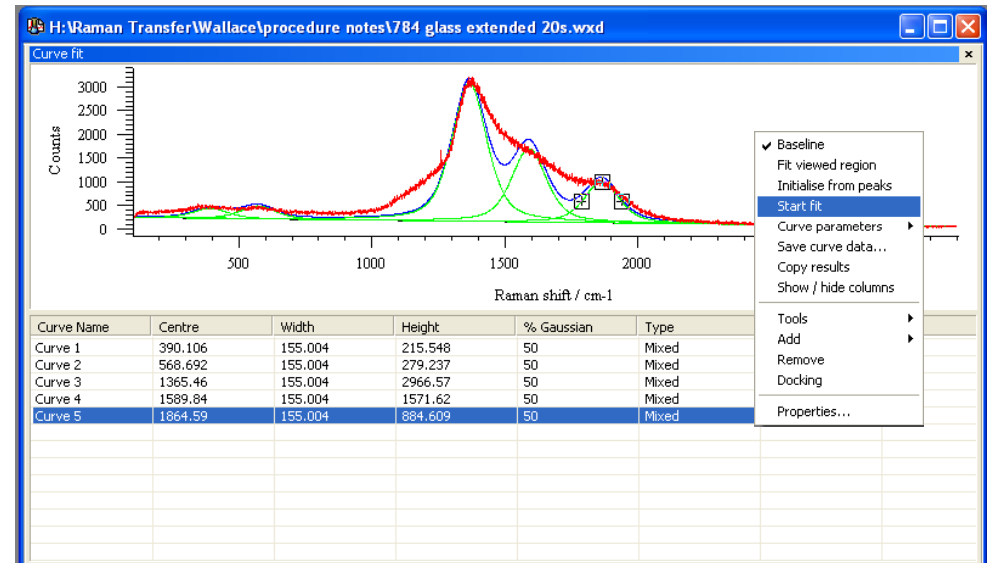
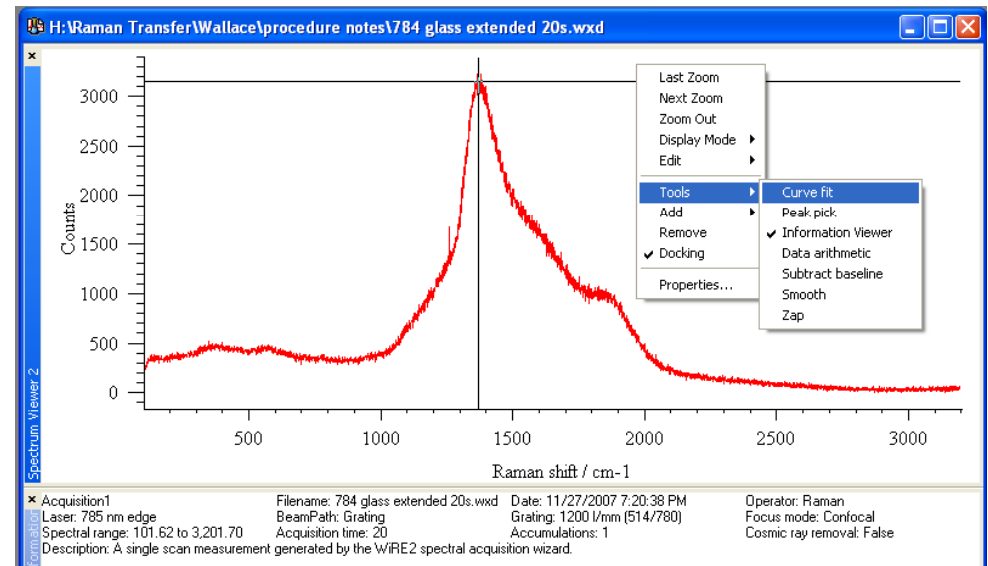


1. Zoom on peaks of interest.
2. Activate Curve Fit from the tool bar, drop down menu **Tools>Curve Fit**, or right click on the active spectra.
3. Click once at the peak of each curve to add peak. Options can be set with the **Properties** dialog available by right clicking on the **curve fit window**.
4. Select **Start fit**

Use **Fit viewed region** with zoom to limit the peaks fit.

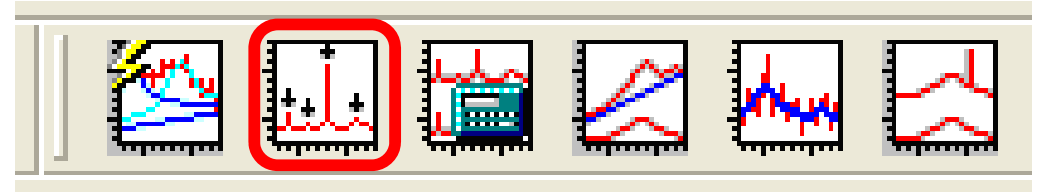
Use Initialize from peaks for simple data sets.

Use **Curve parameters** to Save or Load curves for routine analysis or mapping.

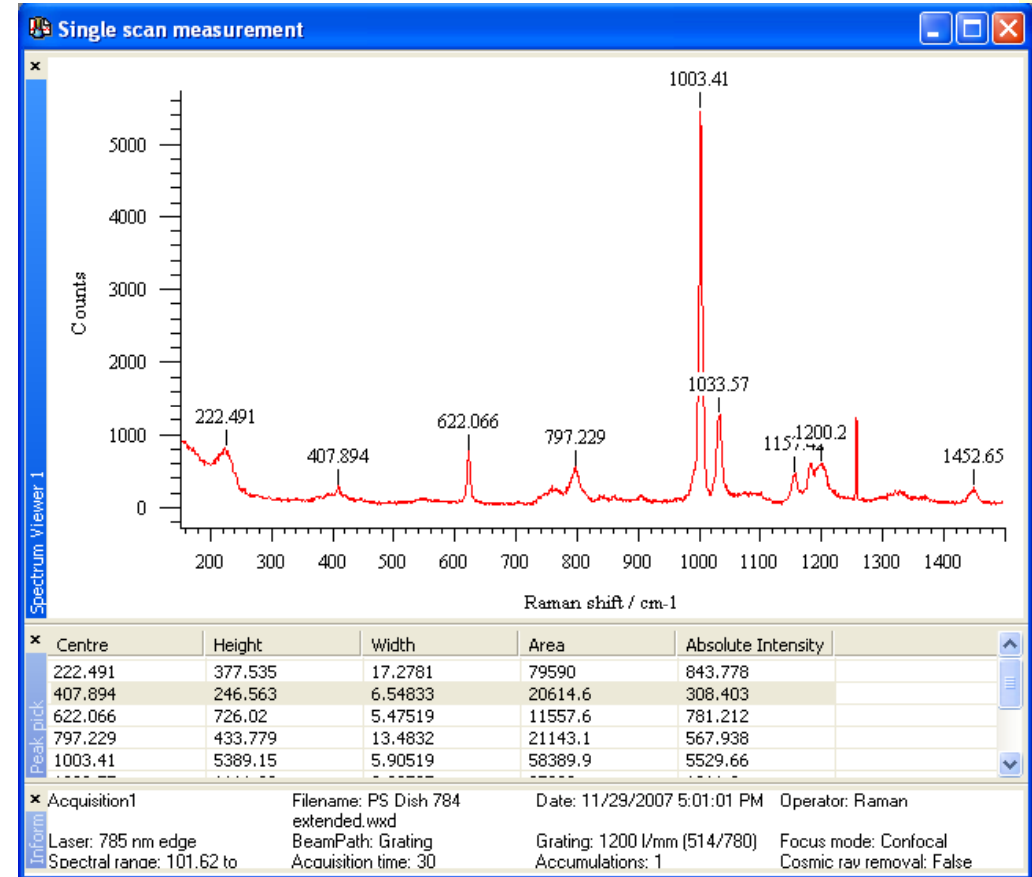


Peak Pick

Curve Fit Peak Pick Arithmetic Baseline Smooth Zap



1. Activate Peak Pick from the tool bar, drop down menu *Tools>Peak Pick*, or right click on the active spectra.
2. Select *Autoset threshold > Whole Spectrum* for all peaks or *Autoset threshold > Single Peak* to select single peaks with the cursor
3. To fit more or less peaks, use the *Properties* dialog available by right clicking on the **peak pick window**.



Peak pick Properties

Peak Picking | Automatic Thresholding | Columns

Peak Detection
 Detection Method: Slope
 Smooth Points: 5
 Minimum Slope: 0.700882

Peak Thresholds
 Height: 95
 Absolute Intensity: 0
 Area: 0
 Peak Interpolation Fraction: 0.75

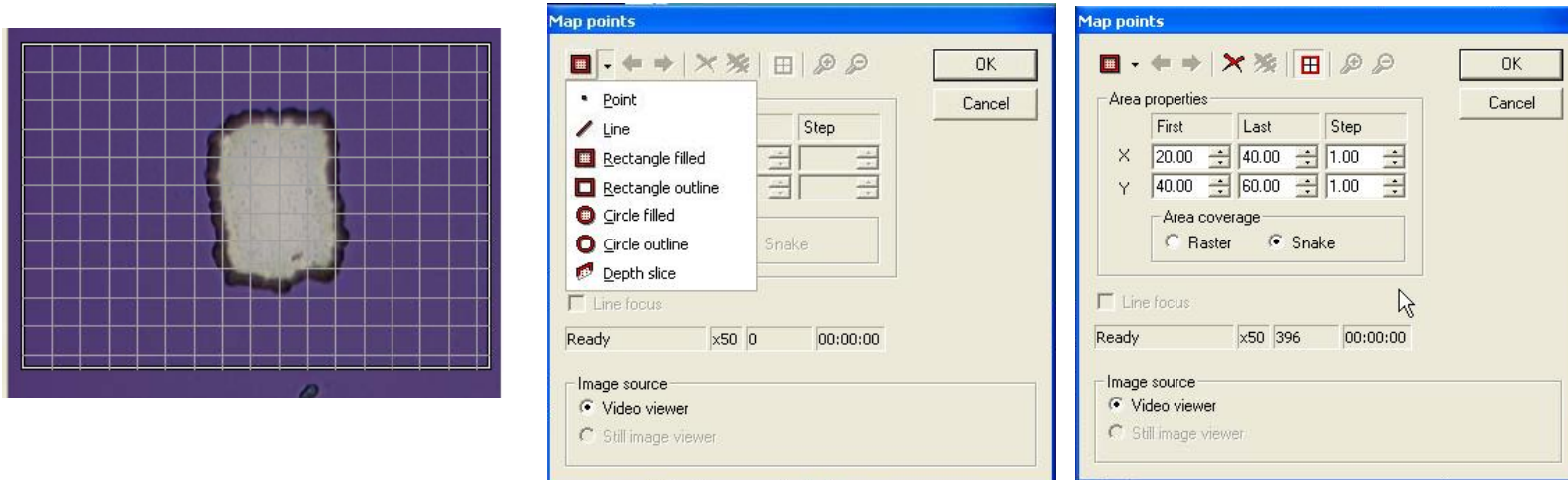
OK Cancel Apply

Decrease for more peaks

Increase for fewer peaks

Select Apply to preview, OK to accept

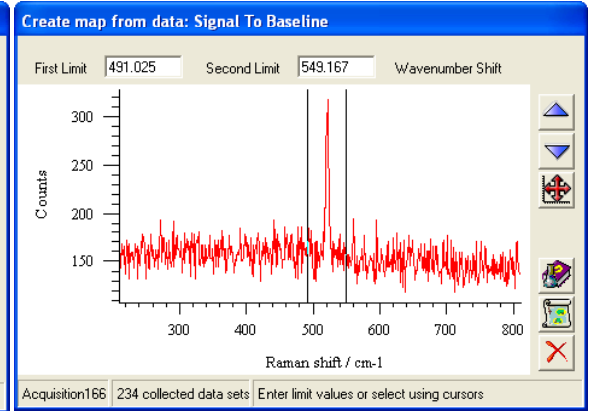
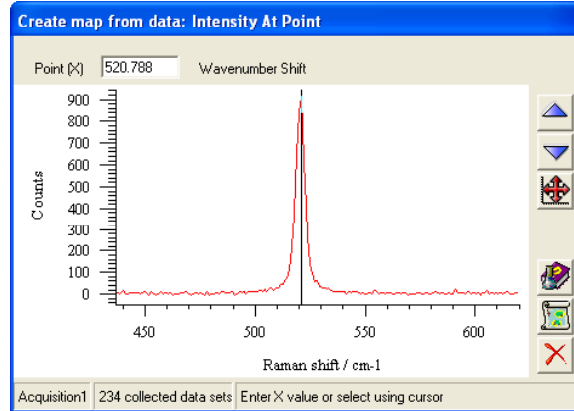
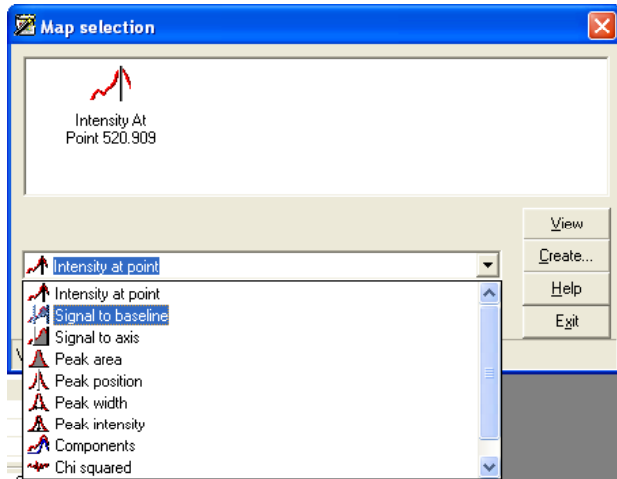
Mapping on Samples



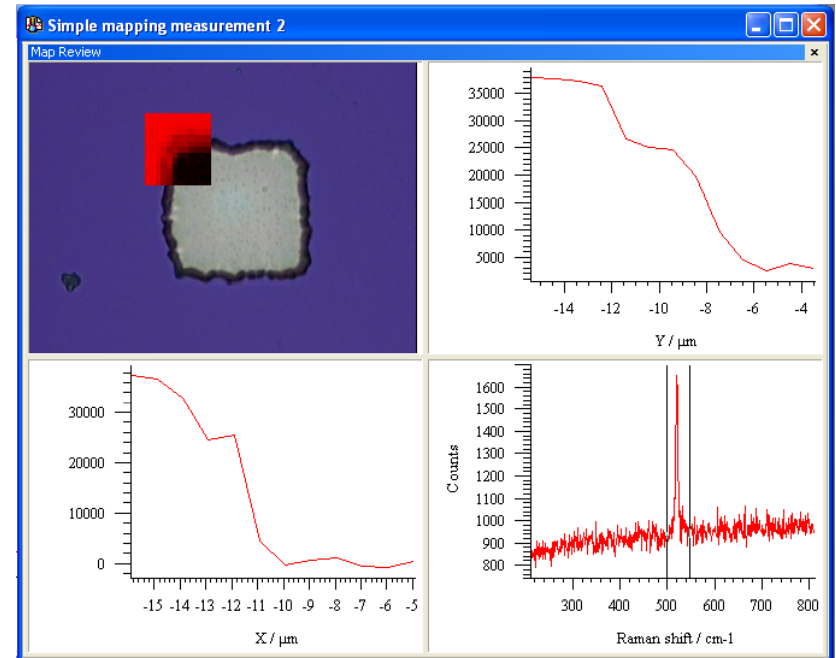
Mapping selections, parameters for “Rectangle filled” shape and its video image.

1. Use **Measurement>New>Map acquisition** to start a new mapping measurement and to setup acquisition parameters for your experiment.
 1. Select mapping area in video image under the BF or DF mode
 2. Select mapping shape, dimensions, and steps
 3. Set experimental conditions for spectra acquisition (Use pinhole for 785 nm)
 4. Set file name (**Spectral acquisition setup > File**)
2. Activate Raman collection channel by switching the turrets to (1/2) .
3. Use **Measurement>Run** or the **Run** button to collect mapping spectra.

Mapping on Samples

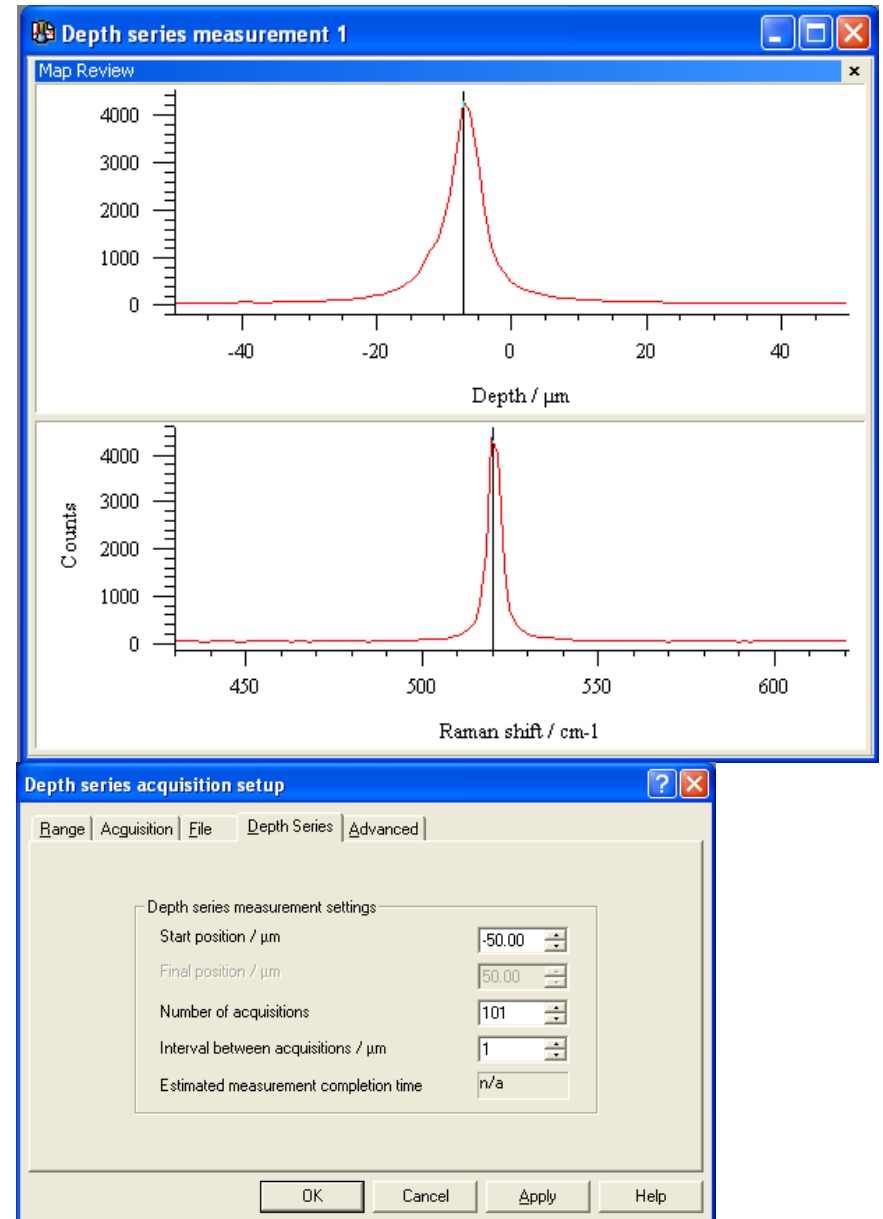


4. Use *Analysis>Mapping review* to analyze data and create 2D images.
5. Select an appropriate method and click on “Create...”
6. Use the cursor to fit data (e.g., peak position for intensity at point) and click on map icon to accept. Click up/down to cycle through spectra.
7. Click on “View” to view the 2D image. Click on any pixel to show the spectrum on the pixel and the intensities of the peak along x and y directions



Depth profile

1. Use **Measurement>New>Depth series acquisition** to start a new mapping measurement and to setup acquisition parameters for your experiment.
 1. Select Start position, number of acquisitions, and interval
 2. Set experimental conditions for spectra acquisition (Use pinhole for 785 nm)
2. Activate Raman collection channel by switching the turrets to (1/2).
3. Use **Measurement>Run** or the **Run** button to collect mapping spectra.
4. Use **Analysis>Mapping review** to analyze data and create 2D images.



Shutdown Procedures

- Close the WiRE 2.0 Software.
- Shut down the microscope and peripherals (lamp and xyz stage controller).
- Turn off the 785 nm diode laser (Key).
- Slowly ramp down power of 514 nm argon ion laser to minimum power and switch key on the small box.
- Logoff current user.
- Sign out in blue 'Leica Microscope' logbook.
- Logout time from the NTUF website.

Appendix 1 Primary Objectives

50X 0.80NA
0.5 mm working
distance



514 nm excitation without/with pinhole at 15 mW (50x/0.8 NA, 1800 l/mm)

Standard confocality – 50 um slit and 7 pixels CCD area – 9000 -11000 counts

High confocality – 20 um slit and 2 pixels CCD area – 3500 – 4500 counts

785 nm excitation without pinhole at 10% output power (50x/0.8 NA, 1200 l/mm)

Standard confocality – 65 um slit and 25 pixels CCD area – 17000 -19000 counts

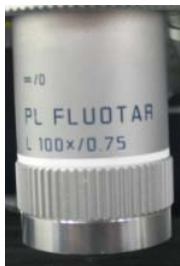
High confocality – 20 um slit and 2 pixels CCD area – 1000 – 1500 counts

785 nm excitation with pinhole at 100% output power (50x/0.8 NA, 1200 l/mm)

Standard confocality – 65 um slit and 25 pixels CCD area – 10000 -11000 counts

High confocality – 20 um slit and 2 pixels CCD area – 1000 – 1300 counts

100X 0.75 NA
4.7 mm working
distance



514 nm excitation without/with pinhole at 15 mW (100x/0.75 NA, 1800 l/mm)

Standard confocality – 50 um slit and 7 pixels CCD area – 3000 – 3500 counts

High confocality – 20 um slit and 2 pixels CCD area – 1000 – 1500 counts

785 nm excitation without pinhole at 10% output power (100x/0.75 NA, 1200 l/mm)

Standard confocality – 65 um slit and 25 pixels CCD area – 10000 -12000 counts

High confocality – 20 um slit and 2 pixels CCD area – 1000 -1200 counts

785 nm excitation with pinhole at 100% output power (100x/0.75 NA, 1200l/mm)

Standard confocality – 65 um slit and 25 pixels CCD area – 4500 -5000 counts

High confocality – 20 um slit and 2 pixels CCD area – 1000 -1200 counts

Please notify staff if the signal is significantly lower than above.

Appendix 1 Additional Objectives

100X 0.85NA
0.33 mm working
distance



514 nm excitation without/with pinhole at 15 mW (100X/0.85NA, 1800 l/mm)

Standard confocality – 50 um slit and 7 pixels CCD area – 8000 -10000counts

High Confocality – 20 um slit and 2 pixels CCD area – 3500 – 4500 counts

785 nm excitation without pinhole at 10% output power (100X/0.85NA, 1200 l/mm)

Standard confocality – 65 um slit and 25 pixels CCD area – 10000 -12000 counts

High confocality – 20 um slit and 2 pixels CCD area – 1000 -1200 counts

785 nm excitation with pinhole at 100% output power (100X/0.85NA, 1200 l/mm)

Standard confocality – 65 um slit and 25 pixels CCD area – 4800 -5000 counts

High confocality – 20 um slit and 2 pixels CCD area – 1000 -1200 counts

10X 0.30NA
11 mm working
distance



514 nm excitation without/with pinhole at 15 mW (10X/0.3NA, 1800 l/mm)

Standard confocality – 50 um slit and 7 pixels CCD area – 400-500 counts

785 nm excitation without pinhole at 10% output power (10X/0.3NA, 1200 l/mm)

Standard confocality – 65 um slit and 25 pixels CCD area – 600 -700 counts

785 nm excitation with pinhole at 100% output power (10X/0.3NA, 1200l/mm)

Standard confocality – 65 um slit and 25 pixels CCD area – 100 -200 counts



100X 1.30NA

0.22 mm working distance

Appendix 2 Optimize your data

To improve signal to noise ratio:

- a) Increase exposure time
- b) Increase number of accumulations

To eliminate strong background due to autofluorescence:

- a) Change excitation wavelength
- b) Quench fluorescence by exposing sample to incident laser light for a period of time
- c) Decrease laser power

To avoid saturated signal:

- a) Reduce laser power
- b) Reduce exposure time

To avoid laser ablation on the samples:

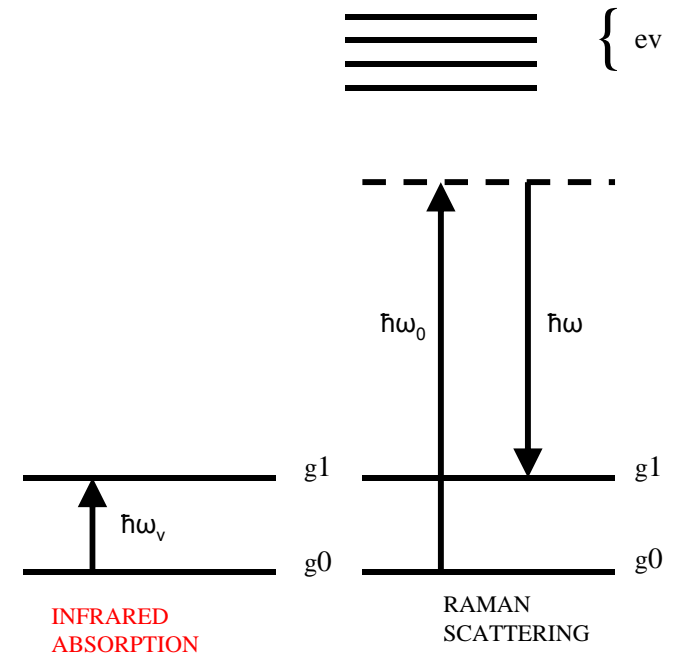
- a) Use a lower magnification objective
- b) Defocus the laser spot
- c) Use a different laser
- d) Decrease laser power

Appendix 3: What is Raman Scattering?

Raman scattering is a fundamental form of molecular spectroscopy. Together with infrared (IR) absorption, Raman scattering is used to obtain information about the structure and properties of molecules from their vibrational transitions.

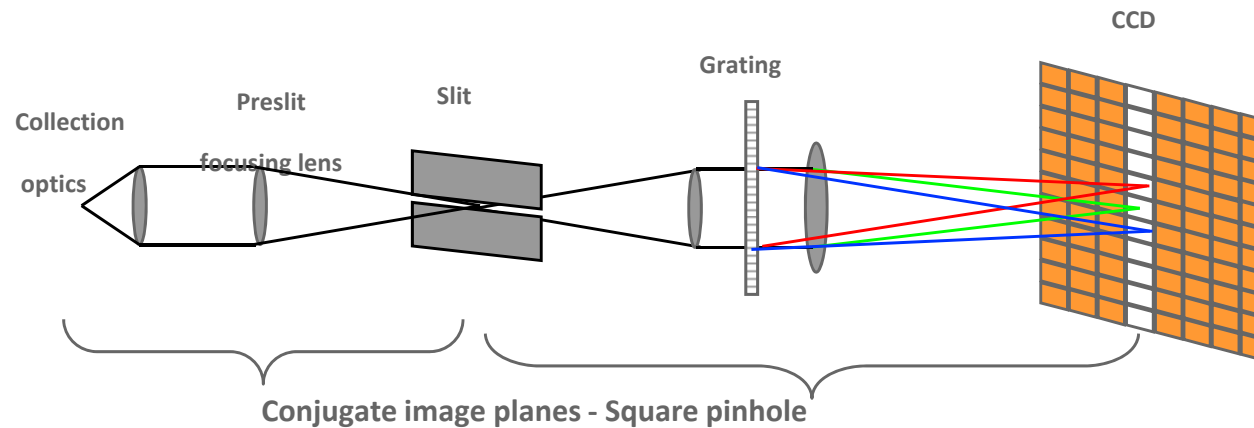
Infrared absorption arises from a direct resonance between the frequency of the IR radiation and the vibrational frequency of a particular normal mode of vibration. The IR photon encounters the molecule, the photon disappears, and the molecule is elevated in vibrational energy when the property of molecule involved in the resonant interaction between the dipole moment of the molecule and its vibrational motion.

In contrast, Raman scattering is a two-photon event. In this case, the property involved is the change in the polarizability of the molecule with respect to its vibrational motion. The interaction of the polarizability with the incoming radiation creates an induced dipole moment in the molecule, and the radiation emitted by this induced dipole moment contains the observed Raman scattering. The light scattered by the induced dipole of the molecule consists both Rayleigh scattering and Raman scattering. Rayleigh scattering corresponds to the light scattered at the frequency of the incident radiation, whereas the Raman radiation is shifted in frequency, and hence energy, from the frequency of the incident radiation by the vibrational energy that is gained (Stokes Raman) or lost (antiStokes Raman) in the molecule.



Energy diagram for infrared absorption and Stokes Raman scattering for a vibrational transition from g_0 to g_1 . The scattering photon energy, $\hbar\omega$, is shifted from the incident laser radiation energy by the infrared vibrational energy, $\hbar\omega_v$, gained by the molecule.

Appendix 4: Renishaw inVia Confocal Raman Microscope



(a) inVia Spectrometer

Includes, triple laser base plate, triple input mirror set, dual gratings on kinematic mount (1800 or 1200 l/mm), spectrometer lens set, manual slit, single Rayleigh filter mount, single laser input mirror set

(b) Renishaw's RenCam deep depletion CCD assy.

Includes front illuminated CCD with Peltier cooling to -70°C , USB interface.

(c) Leica DM IRB microscope interface

Standard Laser Excitation Kits

a) Renishaw 785 nm excitation kit

Includes 785 nm high power laser, 100 cm^{-1} edge filter, laser mounting kit and coupling mirrors, pinhole and interlock cable.

b) Renishaw 514 nm excitation kit

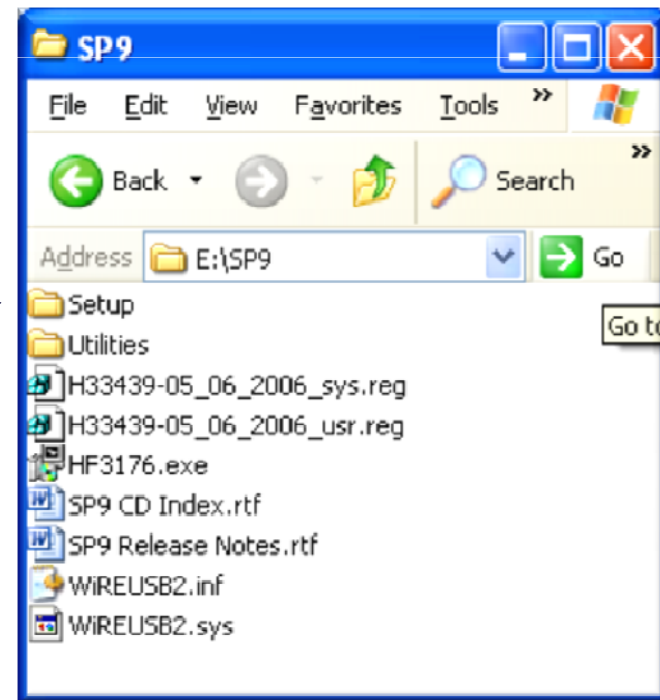
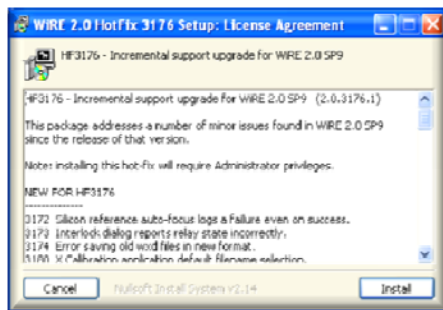
Includes 514 nm laser, holographic notch filter, laser mounting kit and coupling mirror, pinhole, interlock cable and plrf.

Appendix 5: Installing WiRE 2.0 with patch

Software to open and analyze Raman spectra captured at the NanoTech User Facility is provided upon request. After the standard version of WiRE is installed, a HotFix patch is applied to allow advanced tools.

Note: On Vista machines, WiRE 2.0 will only open data. Advanced data analysis tools are not available.

- 1) Transfer "SP9" folder to computer
- 2) Run "Setup.exe" in SP9\Setup folder
- 3) Run "HF3176.exe"



- 4) Double click on Registration Entries H33439-05_06_2006_sys.reg and H33439-05_06_2006_usr.reg
Click "Yes" to modify registry

