Experiment 6
Raman Spectroscopy

I. Raman spectroscopy

Spectroscopic analytical methods are based on measuring the amount of radiation produced or absorbed by molecules – that is the interaction between the radiation and matter.

Infrared (IR) and Raman spectroscopy both are used to probe the characteristic vibrations which can provide information about chemical structure of chemical compounds. The two techniques are usually regarded as complementary. In the most basic terms, IR and Raman spectroscopies are actually quite different in terms of the origins of the phenomena and how they are observed and measured.

The IR spectrum is obtained by measuring the absorption of IR radiation caused by vibrational transitions in

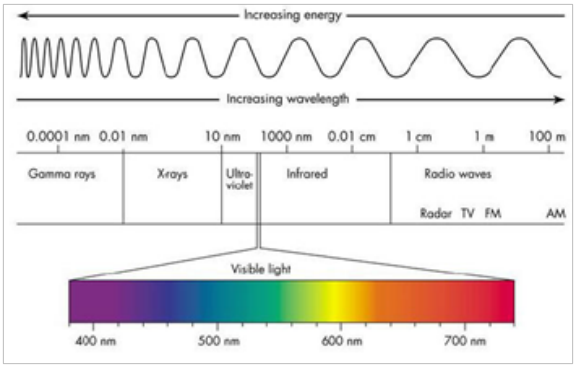

Figure 1. Electromagnetic spectrum.

which a photon whose energy matches a vibrational energy spacing $h\nu_0$ is absorbed. This is a one photon process. The energies of the IR radiation (Figure 1), 12900 to 10 cm^{-1}, are on the order of energies of vibrational transitions. Hence IR is a branch of vibrational spectroscopy. Vibrational transitions occur because a molecule has a multitude of quantized energy levels or vibrational states associated with the bonds that hold the molecule together. The IR range is divided into mid-IR (MIR) with the frequencies in the range of 400–4000 cm^{-1}. The region below 400 cm^{-1} is classified as the far-IR (FIR) and the region between the MIR and the visible part of spectrum is known as near-IR (NIR).

When radiation interacts with the matter in addition to the absorption, emission, or luminescence of electromagnetic radiation by atoms and molecules several additional processes can occur. The radiation can, for example, be refracted, reflected, or scattered. While the first two do not find many analytical applications, the scattering of radiation forms the basis for several analytical techniques including Raman spectroscopy.

In Raman experiment the light is scattered from a sample after irradiation from a high intensity monochromatic source, typically a laser. Raman scattering is a two-photon process in which one photon is absorbed and another is emitted essentially simultaneously. Most photons collected in the Raman measurement results from elastic scattering in which the scattered radiation is of the same frequency as the incident radiation. This is known as Rayleigh scattering.

In the classical explanation of Raman scattering, the incident electromagnetic radiation creates an oscillating

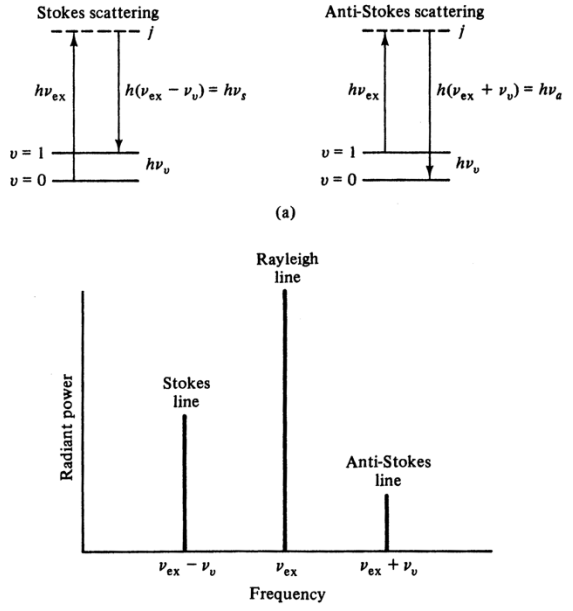

Figure 2. Energy level diagram illustrating Raman scattering (a) and resulting Raman spectrum (b).

induced dipole moment μ with the magnitude dependent on the product of the field and polarizability of the molecule

$$\mu = \alpha E \quad (1)$$

where E is the magnitude of the electric vector of the electromagnetic field that acts on a molecule and α, the polarizability. The induced dipole constitutes the source of the radiation detected as the scattered light. The electric field produced by the polarized molecule oscillates at the same frequency as the passing electromagnetic wave so that a molecule acts as a source sending out radiation of that frequency in all directions. The intensity of the scattering depends on the square of the induced dipole moment, and thus on the square of the polarizability.

From the quantum mechanical point of view light scattering can be regarded as transitions between energy levels. A molecule in the ground state absorbs an incident photon and reaches a virtual excited state which does not correspond to any particular quantized energy level of the atom or molecule. The molecules instantaneously return to the ground state emitting a photon of the same energy that was absorbed (Rayleigh scattering).

A small fraction of the scattered radiation results from inelastic scattering, where the emitted radiation is shifted to higher or lower frequencies compared to the incident radiation. The difference in frequency is equal to a natural vibration frequency of the molecule's ground electronic state and is caused by vibrational transitions that occur during the energy transfer of scattering process. This is referred to as Raman effect (Figure 2), a phenomenon first observed by Sir C. V. Raman in 1928. Usually photons of energy $h\nu_{ex}$ (incident radiation) are absorbed by molecules in their ground vibrational state ($v = 0$). If the molecule returns to vibrationally excited state (not to the original vibrational ground state) it reemits radiation of lower frequency, $h(\nu_{ex} - \nu_v)$, than the incident radiation and give rise to Stokes lines. Several Stokes lines are normally observed in Raman spectrum, corresponding to different vibarations in the molecule. Correspondingly, Raman scattering of molecules in vibrational excited state produces anti-Stokes lines with frequencies $h(\nu_{ex} + \nu_v)$ shifted toward higher frequencies. The Stokes frequency is commonly labeled ν_s and anti-Stokes ν_a. A spectrum of the scattered radiation (Figure 3) consists of a relatively strong component with frequency unshifted with respect to the incident radiation (Rayleigh scattering) and the two components of the Raman

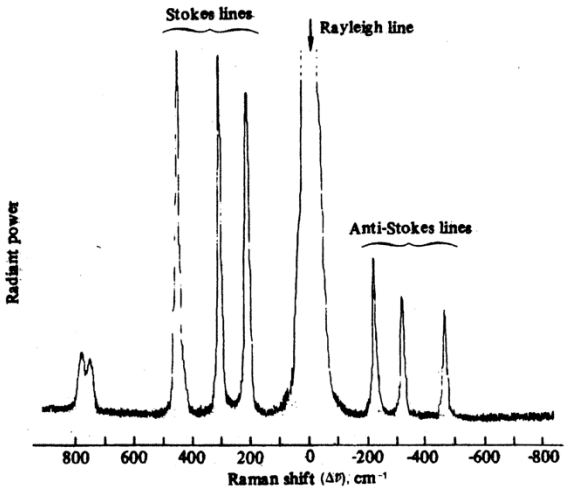

Figure 3 Raman spectrum of pure carbon tetrachloride. The Raman shift is the difference in wavenumbers between the Rayleigh line and the Raman line. From B. J. Bulkin, *J. Chem. Educ., 46,* A781 (1969).

spectrum: the Stokes and anti-Stokes lines. Under normal laboratory conditions most molecules are initially in the lowest vibrational level producing more intense Stokes lines compared to anti-Stokes and usually only the former are considered in chemical analysis.

The frequency shifts between the incident radiation and the Raman scattered radiation (Stokes and anti-Stokes lines) corresponds to vibrational energy levels. Hence we expect Raman spectra to yield information similar to IR spectra.

Both IR and Raman spectroscopies are derived from vibrational transitions and the information content will have some commonality. However the spectra are not identical because not all energy transitions are allowed. For an IR vibration to be active that is for it to absorb IR energy, the molecular vibration must induce a net change in dipole moment during the vibration. For a molecular vibration to be Raman-active there must be a net change in the molecular polarizability during the vibration. The result of these different selection rules is that the two sets of spectral data are often complementary. The differences between IR and Raman activity arise from the different properties of the dipole moment and polarizability operators. For molecules with a center of symmetry these differences lead to the **mutual exclusion principle** where there are no IR active transitions in common with Raman active transitions. For example, the symmetric stretching mode of CO_2 is IR inactive because there is no dipole

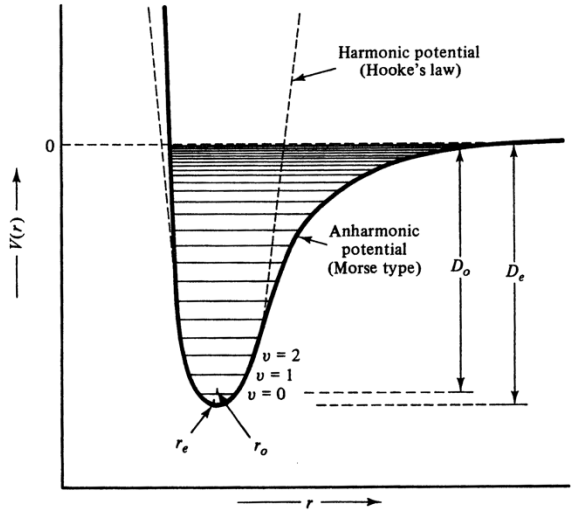

Figure 4. Harmonic and anharmonic potentials for a diatomic molecule.

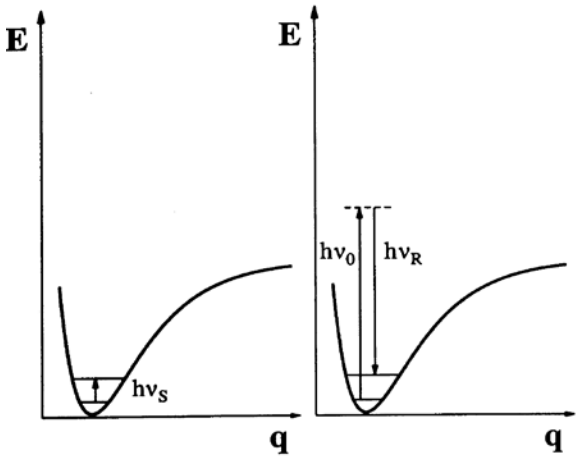

Figure 5. Excitation of a vibrational state in the electronic ground state S_0 observed by IR (left) and Raman (right) spectroscopies.

moment change during vibration. On the other hand, the polarizability varies during the vibration, which leads to Raman activity. For the asymmetric stretch of CO_2, the dipole moment changes during the vibration. However, as the polarizability of one of the C—O bonds increases as it lengthens, that of the other decreases, and overall, there is no change. Thus the asymmetric stretching vibration of CO_2 is Raman inactive. For noncentrosymmetric molecules, there are many cases in which the mutual exclusion principle still holds. However, many other vibrations may be both Raman and IR active. The molecules with no other symmetry elements than the identity operator, which leaves the molecule unchanged, will have vibrations both IR and Raman active. However the intensities observed for the same vibration may be quite different.

The selection rules for IR and Raman also predict that the lines, corresponding to fundamental modes, in both will occur with $\Delta\upsilon = \pm 1$ with much weaker overtone transitions corresponding to $\Delta\upsilon = \pm 2$. If all vibrational modes were harmonic, no transitions involving changes in υ by more than ±1 would be allowed. The effect of anharmonicity is to relax this rule *i.e.* to allow bands caused by $|\Delta\upsilon| > \pm 1$ to be allowed. This give rise to weak overtone ($\Delta\upsilon = 1, 2, 3\ldots$) and combination ($\Delta\upsilon_i = 1, 2, 3\ldots$; $\Delta\upsilon_j = 1, 2, 3\ldots$) bands which appear along with the bands due to fundamental transitions ($\Delta\upsilon = 1$). Combination bands result when the absorption of a photon excites two vibrational modes simultaneously. If the absorption frequencies of the two independent vibrations are ν_1 and ν_2 a combination band is sometimes observed at frequency $\nu_1 + \nu_2$.

Polyatomic molecules in some cases can give more than 3*N*-6 vibrations due to the overtone and combination bands, but usually the number of observed vibrations is fewer. The number of observed bands may be less than predicted if some vibrations are inactive due to the selection rules, if vibrations are degenerate, if they are very weak or cannot be resolved experimentally, or the vibrations may occur outside the range of spectrophotometer.

II. Molecular Vibrations

In a polyatomic molecule each atom has three degrees of freedom – it can move independently along each of the axes of a Cartesian coordinate system. For a molecule consisting of *N* atoms there are 3*N* degrees of motional freedom. Three of these represent translational motion in mutually perpendicular directions (the *x*-, *y*-, and *z*-axes) and three represent rotational motion about *x*-, *y*-, and *z*- axes. The remaining 3*N*-6 degrees of freedom give the number of ways the atoms in a nonlinear molecule can vibrate, *i.e.* the number of vibrational modes. For linear molecules there are 3*N*-5 vibrational degrees of freedom since a linear molecule has two rotational degrees of freedom. All vibrations of a molecule result from superposition of 3*N*-6 (or 3*N*-5 for linear molecules) *normal vibrations*. In each normal vibration all atoms are vibrating with the same phase and frequency.

The simplest vibration of a diatomic molecule consisting of a single, isolated bond can be described in term

of harmonic oscillator. This model is based on Hooke's law

$$F = -k \cdot q \tag{2}$$

where F is the force necessary to move the atoms by a certain distance q from an equilibrium position, k is the force constant which is a measure of the strength of a bond. The potential energy V of a harmonic oscillator is proportional to the square of the displacement.

$$V = \frac{1}{2} k \cdot q^2 \tag{3}$$

The frequency of vibration (in s^{-1}) of a diatomic molecule with the masses m_1 and m_2 is given by:

$$\nu = \frac{1}{2\pi} \sqrt{\frac{k}{m}} \tag{4}$$

Where m is called reduced mass

$$\frac{1}{m} = \frac{1}{m_1} + \frac{1}{m_2} \tag{5}$$

For a diatomic molecule the potential energy V as a function of the internuclear distance r of the atoms from their equilibrium position r_e is shown in Figure 5. In the harmonic oscillator approximation the potential energy function is parabolic in shape (dotted line in Figure 5) and if we use this form of the potential energy to solve the Schrodinger equation we will obtain the quantized energy levels (vibrational energy levels) described by

$$E_\upsilon = h\nu \left(\upsilon + \frac{1}{2}\right) \tag{6}$$

where h is the Planck constant, ν is the fundamental frequency of the particular mode (Eq. 4), and υ is the vibrational quantum number (υ = 1, 2, 3…). The actual variation of the potential energy as a function of the displacement of atoms from their equilibrium position (solid line in Figure 5) fits the parabolic function (harmonic approximation) only near the equilibrium internuclear distance. In practice the *anharmonic* (Moors-type) potential function more closely resembles the potential energy of vibrations in a molecule for all interatomic distances. If the anharmonic potential is used to solve the Schrodinger equation the solution becomes more complex and the approximate solution for the vibrational energy levels is given by

$$E_\upsilon = h\nu \left(\upsilon + \frac{1}{2}\right) + h\nu\chi_e \left(\upsilon + \frac{1}{2}\right) \tag{7}$$

where χ_e is the anharmonicity constant. χ_e is dimensionless, typically 0.002 to 0.02.

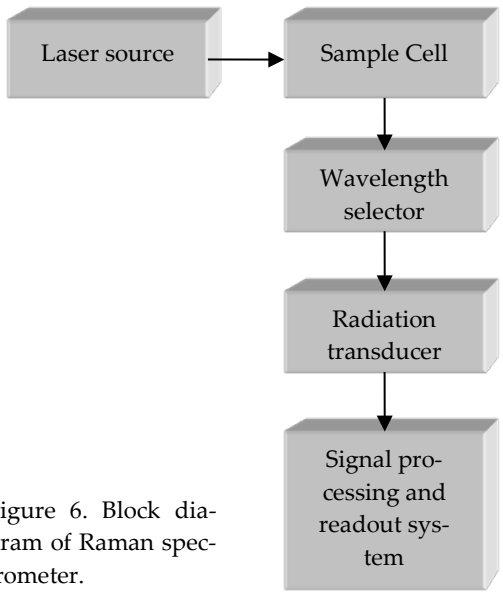

Figure 6. Block diagram of Raman spectrometer.

For many vibrational modes, only a few atoms have large displacements at the rest of the molecule is almost stationary. The frequency of such modes is nearly independent of the rest of the molecule. For example the stretching vibration of carbonyl group in aldehydes and ketones is almost always observed in the range 1650 – 1740 cm^{-1}. Such frequencies are characteristic of functional or structural group involved and are thus known as **group frequencies**. The presence of various group frequencies is of great importance in identifying the molecule. Extensive spectra/structure correlation tables have been developed to allow spectroscopist to assign vibrational bands in a given spectrum to the vibrational modes associated with a certain functional group. Not all bands are useful for identifying functional groups in the structure of organic molecules. In the region from ~400 to 1300 cm^{-1} vibrational frequencies are affected by the entire molecule so their frequencies varies from one molecule to the another containing particular functional group. The modes are useful for distinguishing one molecule from other containing similar functional groups and hence are often known as **fingerprint bands**. These bands find widespread use for identification purposes by comparison with library spectra.

III. Instrumentation

Raman spectroscopy has been greatly influenced by instrumentation development. A typical instrument for

Raman spectroscopy consist of the laser source, a sample cell, a wavelength selector, a radiation transducer, signal processor and readout device.

III-A. Light sources

Before the invention of lasers in 1960, radiation emitted by mercury arc, especially at 435.8 and 404.7 nm, has been used for exciting Raman spectra. Today virtually all Raman instruments use the argon or krypton lasers which provide outputs ranging from blue to red part of the spectrum. Helium-neon (He-Ne) lasers used in early laser-excited Raman spectrometers provide excitation line in the red part of the spectrum, 632.8 nm. NIR Raman spectra are excited mainly with a neodymium-doped yttrium aluminum garnet laser (Nd:YAG), emitting at 1064 nm.

Table 1. Laser excitation wavelengths.

Laser type	Wavelength (nm)
Ne-Ne	632.8
Ar+	488.0, 514.5
Krypton	530.9, 647.1
Nd:YAG	1064

The excitation wavelength for Raman spectrometry has to be chosen carefully. Since the scattering intensity increases as (ν_{ex}^4) and the measured quantity in a Raman experiment is a frequency shift from the laser excitation wavelength rather than absolute wavelength the UV and visible lasers operating at short wavelength should be preferred. However, the high-energy photons generated by the short-wavelength laser often cause photodecomposition of samples as well as fluorescence which is much more intense that the Raman emission from the sample under study. Hence a compromise is often made in selecting the excitation wavelength. The laser used in our laboratory is an Argon-ion laser with a single excitation wavelength at 488 nm.

III-B. Cell configurations

One of the big advantages of the Raman spectroscopy is the possibility of obtaining spectra with solid, liquid, and gas samples using optical materials made of glass and quartz. This is possible since the exciting and scattered radiation is in the visible region transparent to such materials. The most common cells for liquid samples are capillary tubes. Sometimes NMR tubes or a test tube can be used for liquid samples. The latter is used in our setup. Special cells for gas-phase samples are commercially available. Solid samples are also relatively easy to use. Powders can be tamped into an open end capillary. Potassium bromide pellets mounted at 45° to the laser beam are also quite frequently used. For highly absorbing materials such as colored solutions rotating cells have been developed to avoid sample degrading by the lasers. By spinning the sample in the laser beam, intense, localized overheating of the sample is avoided.

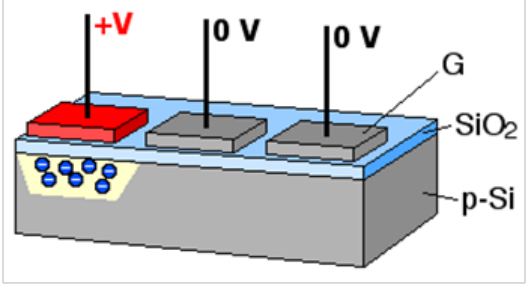

Figure 7. The electrons are collected in potential wells (yellow) created by applying positive voltage at the gate electrodes (G). Applying positive voltage to the gate electrode in the correct sequence transfers the charge packets.

III-C. Wavelength selection devices

Light collected from a sample has to be processed so that the wavelength and intensity information can be extracted from the detected signal. Monochromators block all but a narrow spectral region from reaching the detector. The key component of a monochromator – diffraction grating – is a plane or concave plate that is ruled with closely spaced grooves. The greatest technological difficulty for Raman spectroscopy has been the weakness of the Raman spectral signal compared with the magnitude of the laser excitation wavelength. The solution to this problem how to measure such a low signal in the presence of the dominant signal (Rayleigh line) was to use more than one monochromator. While instruments featured up to three monochromators had the desired optical properties, they were mechanically complex and had very low optical throughput. The very important innovation which virtually eliminated the need for the second and third monochromator was the introduction of laser line rejection filters. Two important types of laser blocking filters are notch filter and edge filters. Notch filters strongly attenuate a narrow spectral region and transmit the rest. In our set a notch filter is employed to attenuate the 488

nm excitation line of the argon-ion laser. Edge filters transmit a spectral region on one side of a given wavelength and block on the other side. The ruled gratings have been replaced with holographic gratings in modern spectrometers. These gratings allow Raman signal to be obtained with better signal-to-noise ratio especially close to laser line. A side benefit of the use of notch filters and holographic gratings and the elimination of the additional monochromators was the reduction in the instrument size. The example is a very compact laser system you are going to use in the lab.

III-D. Transducers

A radiation transducer or photodetector, converts the optical signal into a corresponding electrical signal that can be readily processed by electronic systems. The cooled charge coupled devices (CCD) are the most often used detectors in Raman spectroscopy. A CCD is an example of multichannel array detectors with multiple resolution elements, allowing spatial resolution in one or two dimensions. Such detectors are composed of an array of pixels where each pixel is one resolution element of the array. Array detectors are integrative devices, collecting electrons for as long as the detector is exposed to light. After exposure, the device is sampled to read the signal. A CCD consists of a two-dimensional array of pixels on a silicon chip. Each pixel is composed of metal-oxide (MOS) electrodes, called gates, on top of the chip. A potential well is produced under this gates and stores electrons created by incident photons. The array is read by shifting the electrons from pixel to pixel across the chip into a register. A series of analog amplifiers and analog-to-digital converters (ADC) convert the analog signal into a computer readable format. The recent development of very high performance array detectors with extremely low noise characteristics and high quantum yield represents a major advance in Raman spectroscopy. These parameters are often comparable with those of the photo-multiplier tubes (PMTs) which were the preferred transducers in Raman spectrographs. However, elimination of the single channel detector such as PMTs eliminated the need to scan the monochromator mechanically resulting in a high-efficiency spectrographic system without moving parts. Microcomputers are also used for signal processing including averaging, digital enhancing, smoothing, and spectral matching and for presenting the final data.

IV. Instrument operation

SAFETY PRECAUTIONS

Never look directly into the main laser beam. Never sight down a beam into its source.

Do not place reflective objects in the laser beam.

Always wear LASER SAFETY GOGGLES while working with the Raman instrument.

IV-A. Laser Operation

The following operations are to be done with TA supervision. Please do not attempt to perform it without TA knowledge.

Start UP

1. Connect the remote interface controller (Figure 8).
2. On the power supply, turn DISCHARGE ON key switch to OFF (Figure 9).
3. With a flat-blade screwdriver open the shutter at the front of the laser head.
4. Ensure that the “INTERLOCK” switch is in the “LOCAL ON” position on the remote interface (Figure 8).
5. Plug the AC power cord into a suitable outlet. The POWER ON indicator should light.
6. Turn DISCHARGE ON key switch to ON.
7. At the remote interface, push ON under TUBE CURR. After approximately a 40 seconds delay, the laser will light. The laser initially operates in the IDLE mode, with a current setting of approximately 4.0 amps.
8. For normal operation, press the RUN button on the remote interface front panel. Use the up arrow and down arrow to increase or decrease the current. (When the laser is at the extreme low or high end of a setting, hold down the arrow control for

several seconds to bring the digital circuitry back "on scale". The meter reading then changes.)

Shut Down

1. At the remote interface press IDLE after approximately one minute press OFF under TUBE CURR.
2. Turn the key switch to OFF.
3. Wait until the laser cools down and unplug the AC power cord.

IV-B. Collecting spectra

1. Power on the camera (CCD detector), i.e. switch the power supply ON. **Note:** The camera must be turned on before the WinSpec/32 is opened.
2. Start the application software – WinSpec/32.

There are two data collecting modes, **Focus** and **Acquire**. In most applications the **Focus** mode is used to establish the optimum performance (i.e. to maximize the signal) and the actual data collection is performed

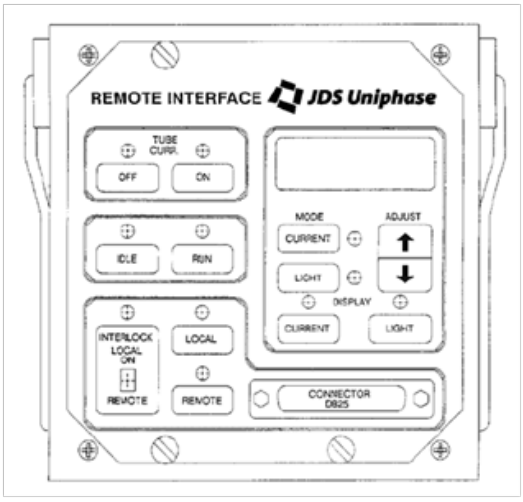

Figure 8. Model 2500 Remote Interface Controller Front Panel.

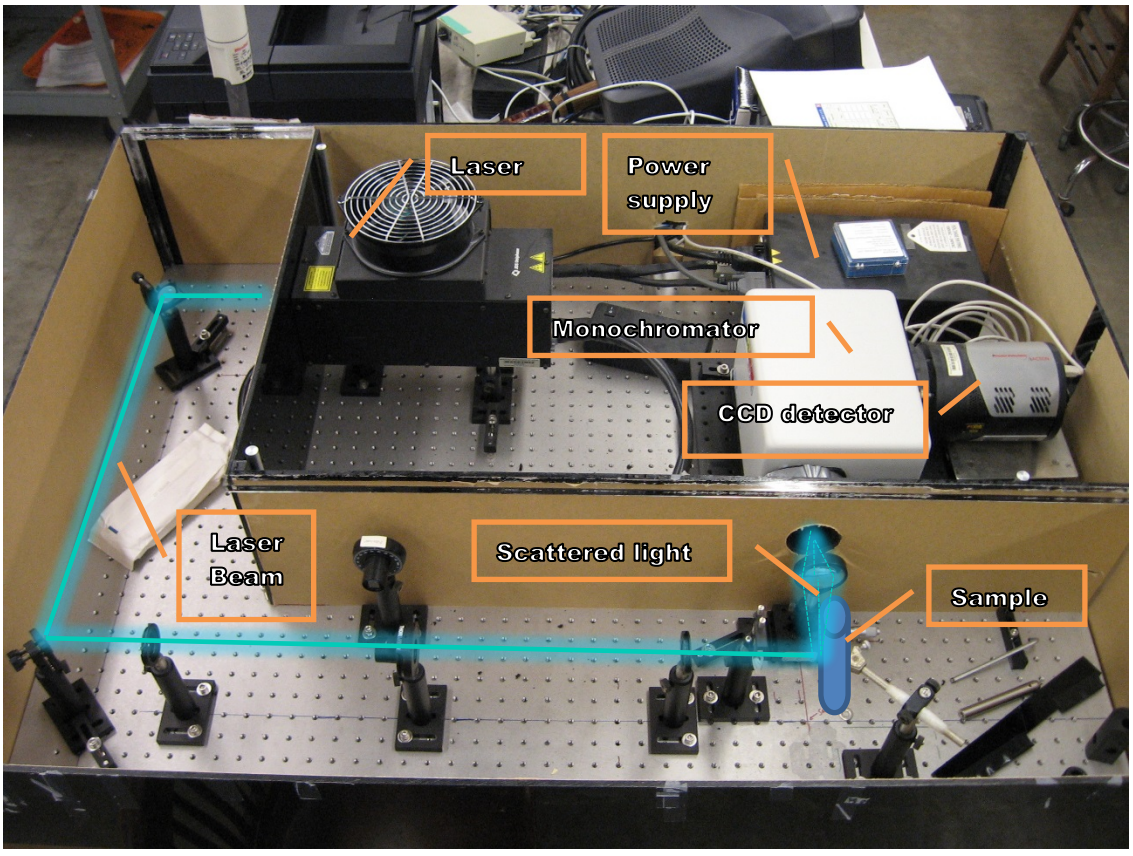

Figure 9 Laser setup.

in the **Acquire** mode. In the focus mode no data is stored. The focus or acquire modes can be started by clicking **Focus** or **Acquire**, respectively, in the Acquisition menu. The parameters controlling the experimental procedure can be changed via **Experiment Setup** dialog box available in the Acquisition menu. Once all the parameters are set this dialog box allows starting the focusing mode or data acquisition by clicking the appropriate buttons.

IV-B-a. Settings for Focus mode.

You should follow the steps below at least once, i.e. before you collect your first spectrum. However, if you already changed the setting described in this section you can use the focus mode to quickly check your sample before acquiring the actual spectrum. In this case it is sufficient only to adjust the exposure time to 0.5 seconds (or lower) and the number of accumulations to 1 on the Main tab and perhaps uncheck the background correction on the Data Corrections tab. Once these adjustments are done in order to start the Focus mode select *Focus* from *Acquisition* menu. To stop the *Focus* mode click the red *Stop* icon on the Toolbar. Otherwise follow the steps below.

1. Open the **Experiment Setup** dialog box from the Acquisition menu (Figure 10). *Change only the settings indicated below leaving all others unchanged.*
2. On the **Main** tab page set the following parameters
   a. **Exposure time**: 0.5 seconds
   b. **Use Region of Interest**: selected
   c. **Accumulations, Number**: 1

   The exposure time defines how long the CCD's pixel senses the intensity of light (photons) falling on its collection area. Since the amount of charge stored in each pixel's "well" is proportional to the number of photons it collects - the larger the exposure time the greater the signal and we see higher peaks on the computer screen. The option Accumulations defines the number of spectra which will be collected and averaged. This is the simplest way to enhance the S/N (signal-to-noise) ratio.
3. On the **ROI setup** tab page specify the Name and
   a. **Spectroscopy mode**: selected
   b. Click on **Full** to load the full size of the chip into the edit boxes. The **Start, End**, and **Group** should show 1, 1340, and 1, respectively.
4. On the **Data Corrections** tab page all the correction functions should be **OFF** with the exception of the **Cosmic Ray Removal** which should be set to **Temporal**. This will help remove highly localized spikes.

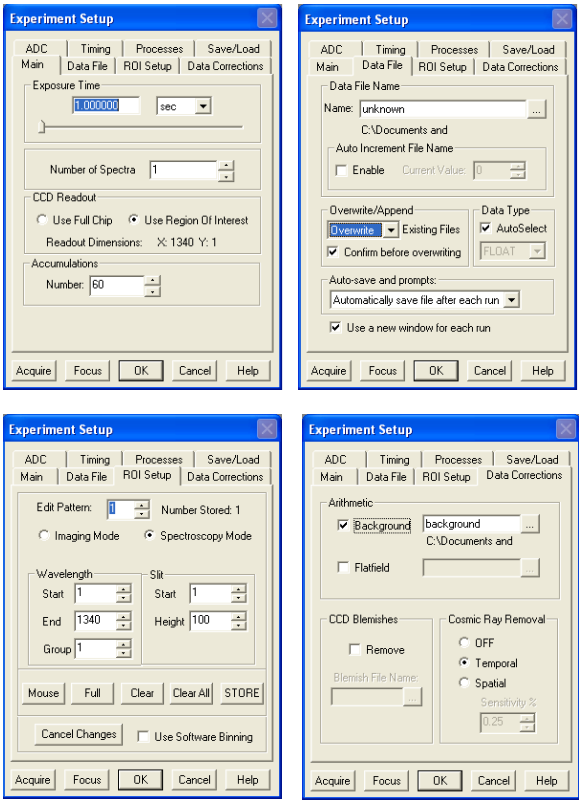

Figure 10. Experiment Setup dialog box.

IV-B-b. Settings for Acquire mode.

It is assumed that you applied all the changes in the **Experimental Setup** dialog box mentioned in the section Setting for the Focus mode (IV-B-a). Modify only the settings indicated below leaving all others unchanged. If you already applied also the setting below, for example you acquired a spectrum before and you want to collect another spectrum, perhaps for a different sample with the same settings follow the steps in the next section, IV-B-c. Otherwise if this is the first time you are acquiring a spectrum follow the steps below.

1. Open the **Experiment Setup** dialog box from the Acquisition menu (Figure 9).
2. On the **Main** tab page set the following parameters
   a. **Exposure time**: 1 second
   b. **Accumulations, Number**: 60

   If the signal is very weak you can increase the number of accumulations. This will improve S/N ratio not the intensity, but it might be easier to resolve the weakest peaks.
3. On the **Data File** tab make the following selections

c. **Auto Increment File Name**: OFF
d. **Overwrite/Append Existing Files**: Select Overwrite (data file will overwrite an existing file having the same name).
e. **Confirm before overwriting**: Checked.
f. **Data type**: AutoSelect should be checked.
g. **Auto**-save and prompts: Select **Ask whether to save unsaved files.**
h. **Use a new window for each run**: Checked.
i. Click on the button to the right of the **Name** field. This will open a browse box. Select directory where you want the stored file to go or create a new directory for your files. The latter is preferable. You can enter a file name in the **Name** field, but remember to save the files under different names when you close/save them. Otherwise you will overwrite all the old spectra you collected with the latest file.
j. Click **OK**

IV-B-c. Acquiring a sample spectrum

You need to set up the parameters described in sections IV-B-a and IV-B-b before acquiring a spectrum.

1. Block the laser beam and put the test tube containing an analyzed solution in the sample holder. ***Exercise extreme caution working with the laser beam. Avoid direct eye exposure. Do not stare directly into the laser beam***
2. Place the test tube in the sample holder.
3. Open the laser beam and make sure the sample is positioned properly.
4. Open the **Experiment Setup** dialog box from the Acquisition menu (Figure 10) and set
   a. **Exposure time**: 0.5 seconds
   b. **Number of accumulations**: 1
5. Click **Focus** button. While observing the displayed data, adjust the system optics and the position of the test tube with the sample for the best possible spectrum (peaks should be as higher and narrow as possible).
6. If the spectrum is acceptable or you cannot improve it any more click the red **STOP** icon on the toolbar to exit the focus mode.
7. Open the **Experiment Setup** dialog box from the Acquisition menu (Figure 9) and set
   c. **Exposure time**: 1 seconds
   d. **Number of accumulations**: 60
8. Click **Acquire** button to begin collecting the data. Alternatively, you can click **OK** start collecting data later by selecting **Acquire** from the **Acquisition** menu.

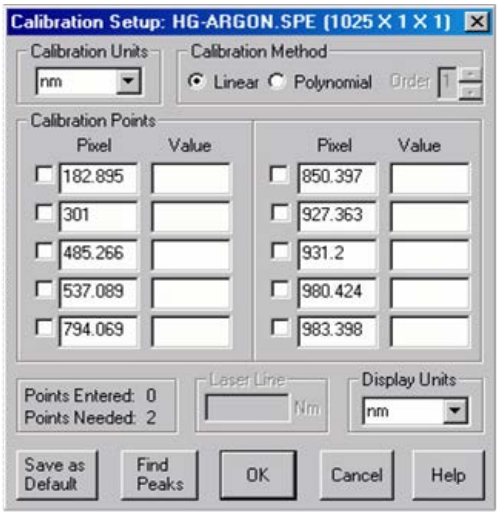

Figure 11. Calibration setup dialog box.

9. Save the spectrum under a new name. Click **Save as** from the File menu. **Note:** Winspce/32 saves each

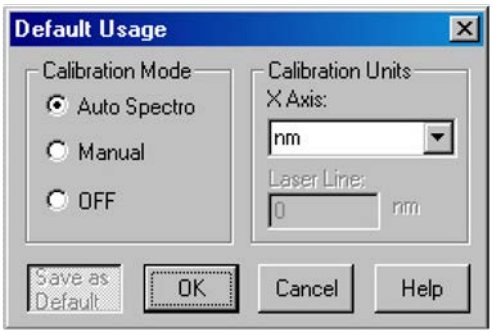

Figure 12. Calibration dialog box.

data file using the name you entered in the **Name** field on the **Data File** tab of the **Experimental Setup** dialog box. If you do not save your spectra under new names next time you will try to collect a spectrum WinSpec/32 will ask you if you want to overwrite the existing file and if you choose YES you will lose the old data.

IV-C. Calibration

First stage of the spectrometer filters out the laser frequency (notch filter), while leaving the rest of the frequencies unaffected, and the second stage spreads the filtered light (grating) onto the detector (CCD array), which then responds uniformly to each frequency. However, the CCD array gives us only information which pixels detected photons. To know the energies

corresponding to the pixels of the CCD detector one needs a calibration of the detector response. The WinSpec/32 software can be calibrated either by using the spectrograph stepper motor position (spectrograph calibration) or by performing a wavelength calibration. The latter is the only option available for our instrument. To selection of the calibration method is controlled by the *Usage* option in the *Calibration* menu. Upon selecting the dialog in Figure 10 will be displayed. The *Auto spectro* selects spectrograph calibration. *Manual* applies wavelength calibration to the active data, and *OFF* selects uncalibrated operation. Do not forget to select the calibration units which in case of vibrational spectroscopy are typically wavenumbers (cm^{-1})

To perform the wavelength calibration at least one spectrum needs to be acquired or loaded. For good calibration results, the spectrum should have well defined peaks for which you know the wavelength. For good calibration the spectrum should have well defined peaks for which you know the wavelength. A calibration requires at least two points defined by pixel and by units. Naturally, the more points you use the more accurate calibration. The ideal calibration spectrum would have one peak at the start of the region of interest, one at the end of the region of interest, and one midway between the end peaks. In real life the ideal spectrum is seldom available but you should try to select the peaks which cover possible the whole region of interest and which are not clustered or close to each other.

For the purpose of our experiment you are going to acquire the spectra of tetrachloromethane and toluene, find the peaks, mark the peaks to be used for calibration, and save the calibration data

1. You will use two solvents tetrachloromethane and toluene for the calibration. Follow section IV-B-b to acquire the spectrum of the first solvent (tetrachloromethane or toluene). Once it is your active (selected) spectrum proceed as follow.
2. Turn off any existing calibration. Click on **Usage** in Calibration menu and change the calibration mode to **OFF** (Figure 11).
3. Click on **Setup** in Calibration menu. This will open the calibration setup dialog box (Figure 12). If a previous calibration had been saved you will see calibration values listed otherwise the calibration points will be blank. The existing calibration values should be erased.
4. Click on **Find Peaks**. An automatic peak finding routine will be performed and 10 highest peaks will be shown in pixel boxes in the calibration setup dialog box.
   Note: The find peak procedure sometimes have problems finding peak maxima or finding peaks at all. If this is the case click where you think the peak maximum is and read the position on the X axis from the status bar. It starts with "X = …" followed by a number. The number indicates the pixel if the calibration if OFF.
5. Select the calibration points you want to use and manually enter the wavelength of selected peaks. Check the selection box to the left of the Pixel box for each peak selected (the selection box can be checked only if the wavelength values is entered). If necessary change also the calibration units. These should be consistent with the units of the calibration points i.e. the values you are entering in the

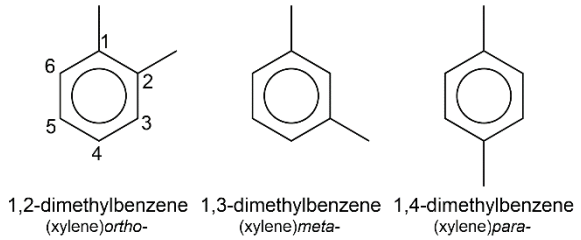

Figure 13. Xylene isomers.

   Value field of the calibration table. Write down the pixel and value for each selected calibration point; keep in mind the calibration units. You will need it when you use the second solvent.
6. Close the dialog box by clicking **OK.**
7. Acquire the spectrum of the second solvent.
8. Click on **Usage** in Calibration menu and change the calibration mode to **OFF**.
9. Find the peak maxima. Click on **Peak Find** in Process menu and try to change sensitivity to find the pixels corresponding to the peak maxima. If this does not work as expected estimate as best as you can the peak maxima by clicking on each peak and write down the corresponding pixel.
10. Click on **Setup** in Calibration menu. The calibration points created for the first solvent should be displayed in the calibration table. Comparing the spectra of the two solvents select a few peaks from the spectrum of the second solvent which are not too close to the peaks selected for the first solvent and which cover the region of interest as uniformly as possible.

11. To complete the wavelength calibration click **OK. Note:** The state of the Save as Default button should be selected. When you press OK the calibration will be stored in Windows Registry and will be automatically applied to all subsequent data until new calibration is performed. **However, it will not be applied to the data loaded from the disk.**

V. Experimental procedure

You will be given an unknown mixture of two xylene isomers. Your goal is to identify which of the two isomers out of the three isomers shown in Figure 12 are present in your sample.

Start the laser following the steps in section IV-A (this part is to be done with TA supervision).

1. Perform the wavelength calibration. This step calibrates the WinSpec/32 software for one position of the grating and allows you to obtain spectra in units of energy rather than pixels.
2. Collect the background spectrum. This operation is very similar to normal data collection procedure and requires acquiring background files.
   a. You need to use exactly the same settings as will be used in data collection therefore before collecting the actual background spectrum you need to follow the steps in section IV-B.
   b. Wait at least 30 minutes after the detector has reached operating temperature to ensure stability.
   c. Remove any sample from the path of the laser
   d. Select **Acquire Background** from Acquisition menu. This will immediately acquire a background file using the Experiment Setup parameters

The procedure called background subtraction allows you to automatically subtract any constant background in your signal. This includes dark noise and also the ambient light which cannot be completely prevented from entering the detector in our setup. The former is caused by thermally induced buildup of dark charge (dark current) in the CCD detector. Additionally, subtraction of the sample matrix signal can also be achieved in this way. In this case you would have to collect and store the spectrum of the sample without the analyte present. To instruct the software to automatically subtract a previously stored background file from each new data acquisition select **Experiment Setup** from Acquisition menu, click on the **Data Corrections** tab and enter the filename in the **Subtract** filename box. This filename is inserted automatically if you acquire a background spectrum through the **Acquire Background** in the Acquisition menu.

3. Record the Raman spectra of the xylene isomers and the spectrum of the unknown mixture. You can use PrtScn and and paste to Paint to save images of your spectra and label the Raman shift (in cm^{-1}) for each peak from 500-1700 cm^{-1}.

VI. Discussion

1. Compare the spectra of the Xylene isomers with the spectrum of the unknown and identify the Xylenes present in your sample.
2. Using tables of characteristic group frequencies try to assign the peaks in your spectra. Which peaks are the best for distinguishing different Xylene isomers? Describe their corresponding normal vibrations.
3. Can you suggest any other instrumental/analytical methods which could be useful in identifying xylene isomers present in an unknown mixture?
4. Thinking back to the GC/MS experiment, which of the two techniques (Raman vs GC/MS) in your opinion is more suitable for analysis of xylene isomers in gasoline sample?