

Title: IR/Raman/SERS Spectroscopy and Selection Rules

Key Words

Infrared (IR) spectroscopy, Raman spectroscopy, Surface Enhanced Raman Scattering (SERS), Selection rules

Object

It aims to learn basic concepts of physical chemistry and nanotechnology by measuring the Raman signal enhancement on colloidal silver nanoparticles (AgNPs). After this experiment, students may be familiar with the fundamental of IR/Raman/SERS spectroscopy and the concepts of light scattering, surface chemistry, and resonance effects.

Introduction

Vibrational spectroscopy has been used as an analytical method to identify organic molecules through infrared (IR)/Raman measurements. IR/Raman spectra produce a vibrational fingerprint unique to each IR/Raman active molecule. Even structurally similar molecules can yield distinct signals. Both methods are complementary in a sense that they rely on different selection rules, so that IR inactive vibrational modes can be active on Raman signals or vice versa. Furthermore, Raman spectroscopy is compatible with aqueous systems, while IR is not because of its intense absorption, which renders Raman appropriate for biological applications.

In 1973, the surface-enhanced Raman scattering (SERS) effect was first introduced by Martin Fleischmann, Patrick J. Hendra and A. James McQuillan at the Department of Chemistry at the University of Southampton, Southampton, UK. The SERS effect was observed from the large increase in Raman signal of pyridine adsorbed on electrochemically roughened silver, and has generated considerable excitement in the physics and chemistry communities. At this time, the detected pyridine concentrations (1.0×10^{-1} M for normal Raman and 6.25×10^{-3} M for SERS measurement) were significantly large when compared with the trace amount of analyte that can be now detected via SERS. In the next year, single-molecule SERS-based detection and identification under the favorable circumstances was feasible through experimental and theoretical studies. Due to the immense enhancement, SERS suggested the cutting-edge application in biological, chemical, medical, military defense, homeland security, environmental settings and pharmacological fields. However, the present SERS based detection applications demand the development in an accurate determination of the magnitude of the signal enhancement. In this regard, experiment at the undergraduate level has been required to raise highly specialized scientists to this rapidly changing field.

In this experiment, students measure IR/Raman/SERS spectra of rhodamine 6G and compare

them in order to understand the fundamental quantum effects of molecular vibrational motions as well as the selection rules behind it. Raman spectrophotometers were employed to examine the surface-enhanced Raman scattering for rhodamine 6G adsorbed on a Creighton colloid. Among the various kinds of SERS-active substrates, silver colloids are well known to result in huge enhancement factors, and proper for single-molecule SERS experiments. The Creighton method has been widely used due to its simplicity, accessibility, relative low cost, and time efficiency.

Background Information

Theory

It is highly recommended to read chapter 13 of reference 6 (or chapter 5 and 13 of reference 7) to learn more about the basic of Molecular spectroscopy: rotational and vibrational spectra. Below following is partially extracted from the chapter 13, explaining the basic principles of Raman and IR spectroscopy.

1. Light-molecule interaction

Hamiltonian of a molecule consists of electronic, translational, vibrational, and rotational terms.

$$\hat{H}_{\text{mol}} = \hat{H}_{\text{elec}} + \hat{H}_{\text{trans}} + \hat{H}_{\text{rot}}$$

Electronic term is for the quantum feature of electrons, but the others are for the quantum mechanical motions of atoms. Those energy values can be measured by spectroscopy with appropriate frequencies of lights. In this experiment, we learn about the vibrational and rotational terms. Interaction of a molecule with light can be described as the response of nuclei and electron charges of the molecule to an oscillating electric field (classical light = electromagnetic waves). Such response entails induced dipole moment oscillating with the frequency of an external electromagnetic wave that is light. Different molecular processes generating such an oscillating dipole moment have characteristic frequencies, because nuclei and electrons move in a fundamentally different time unit.

Nuclei and electrons in a molecule move in a fundamentally different time unit as shown in Figure 1.

- Electron: attosecond (10^{-18}s)

It takes 160 attoseconds for an electron to orbit a hydrogen atom. Therefore, corresponding frequency of the orbit motion is given by

$$\nu = \frac{1}{160 \times 10^{-18}\text{s}} = 6.25 \times 10^{15}\text{s}^{-1}$$

which falls into the visible and ultraviolet region.

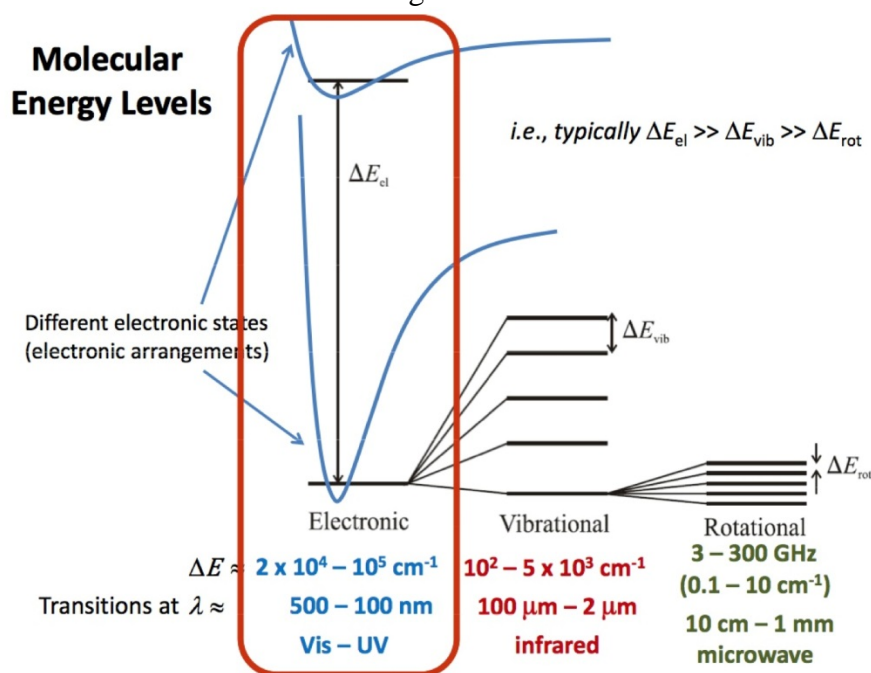


Figure 1. Molecular electronic structures and their related processes

- Nuclei: femtosecond (10^{-15}s)

Molecular vibration and rotation are the result of the motion of nuclei. In general vibrational motion is faster than the rotation, since in the vibration mode nuclei just oscillate around their equilibrium positions with slight deviation. The corresponding frequency of such oscillation is around 10^{-12}s that falls into the infrared region. Characteristic frequency of a rotating molecule strongly depends on the size of the molecule and thus has a broad range from $10^{-9} - 10^{-11}\text{s}$ for large molecules to $10^{-11} - 10^{-13}\text{s}$ for small molecules, which fall into the microwave and far-infrared regions, respectively.

- Bohr frequency

Such a characteristic frequency for a specific molecular motion can be measured through detecting absorption and emission of photons for molecules, which is called the *spectroscopy*. Observed frequency in the spectroscopy, namely the *Bohr frequency* is given by the energy difference between initial and final states as follows.

$$\nu = \frac{\Delta E}{h}$$

or more often in the unit of wavenumber (cm^{-1})

$$\tilde{\nu} = \frac{\Delta E}{hc}$$

By analyzing various characteristic frequencies in spectra, one can obtain structural information of molecules. In addition, it is possible to study chemical dynamics such as

reaction mechanisms by measuring the spectrum as a function of time.

2. Vibrational spectroscopy

Transitions between electronic energy levels are stimulated by or emit ultraviolet, visible, or near-infrared radiation. Vibrational and rotational transitions, the focus of the discussion in this experiment, can be induced in two ways. First, the direct absorption or emission of infrared radiation can cause changes in vibrational energy levels, whereas absorption or emission of microwave radiation gives information about rotational energy levels. Second, vibrational and rotational energy levels can be explored by examining the frequencies present in the radiation scattered by molecules in Raman spectroscopy. About 1 in 10^7 of the incident photons collide with the molecules, give up some of their energy, and emerge with a lower energy. These scattered photons constitute the lower-frequency Stokes radiation (Figure 4). Other incident photons may collect energy from the molecules (if they are already excited), and emerge as higher-frequency anti-Stokes radiation (Figure 4). The component of radiation scattered without change of frequency is called Rayleigh radiation.

A. Vibrational Spectroscopy.

According to **the selection rule, vibrational transition is only allowed between adjacent energy states**. For absorption and emission, $\Delta n = +1$ and $\Delta n = -1$, respectively.

$$\Delta E = h\nu_{vib}$$

where $\nu_{vib} = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}}$ or in the unit of wavenumber (cm^{-1}) $\tilde{\nu}_{vib} = \frac{1}{2\pi c} \sqrt{\frac{k}{\mu}}$

The result is illustrated in Figure 2.

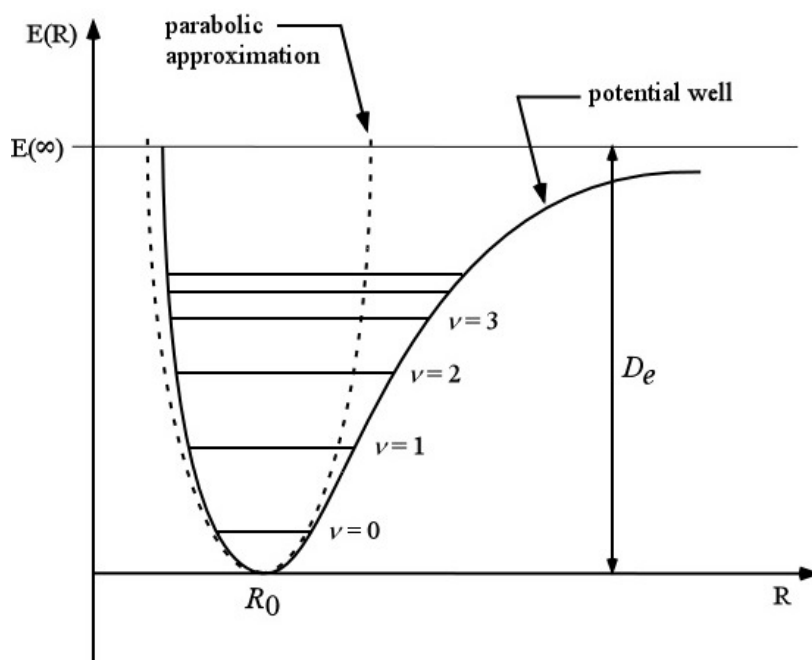


Figure 2. Vibrational energy levels of a molecule.

Since each diatomic molecule has different bond strength, their potential shapes and hence the spring constants $k = \left(\frac{d^2V}{dl^2}\right)_{l=l_0}$ are different from one another. Therefore, their absorption frequencies are unique, which can be used as a finger print of measuring molecules. **Typical vibrational frequency belongs to the infrared region. By measuring the vibrational transition using IR spectroscopy, one may identify a molecule in a given sample, and calculate the force constants.** Due to the selection rule, in the spectrum, you may observe a single line for one type of molecules. **It is called the fundamental vibrational frequency.** However, in real situation, the interatomic potential would deviate from its harmonic approximation. So the selection rule can be violated, and furthermore anharmonic terms should be considered.

B. Anharmonicity

The vibrational terms are only approximate because they are based on a parabolic approximation to the actual potential energy curve. A parabola cannot be correct at all extensions because it does not allow a bond to dissociate. At high vibrational excitations the swing of the atoms (more precisely, the spread of the vibrational wavefunction) allows the molecule to explore regions of the potential energy curve where the parabolic approximation is poor and additional terms in the Taylor expansion of V must be retained. The motion then becomes anharmonic, in the sense that the restoring force is no longer proportional to the displacement. Because the actual curve is less confining than a parabola, we can anticipate that the energy levels become less widely spaced at high excitations (Figure 2).

C. Selection rules and transition moments

The concept of a ‘selection rule’ means a statement about whether a transition is forbidden or allowed. Selection rules also apply to molecular spectra, and the form they take depends on the type of transition. The underlying classical idea is that, for the molecule to be able to interact with the electromagnetic field and absorb or create a photon of frequency ν , it must possess, at least transiently, a dipole oscillating at that frequency. This transient dipole is expressed quantum mechanically in terms of the transition dipole moment, μ_{fi} , between states:

$$\psi_i \text{ and } \psi_f : \mu_{fi} = \int \psi_f^* \hat{u} \psi_i d\tau,$$

where \hat{u} is the electric dipole moment operator. The size of the transition dipole can be regarded as a measure of the charge redistribution that accompanies a transition: a transition will be active only if the accompanying charge redistribution is dipolar. **Only if the transition moment is nonzero or equivalently the vibrational mode involved in the transition**

changes a molecular dipole moment, does the transition contribute to the spectrum. It follows that, to identify the selection rules, we must establish the condition for which $\mu_{fi} \neq 0$ or

$$\frac{\partial \mu}{\partial q} \neq 0,$$

where μ is the dipole moment and q is the coordinate of the vibrational mode.

A gross selection rule specifies the general features a molecule must have if it is to have a spectrum of a given kind. A detailed study of the transition moment leads to **the specific selection rules** that express the allowed transitions in terms of the changes in quantum numbers.

3. The scattering process and vibrational Raman spectroscopy

When light is scattered from a molecule, most scattered photons have the same frequency and wavelength as the incident photons (elastically scattered). However, a little fraction of light (approximately 1 in 10^7 photons) is scattered inelastically, and have different frequencies usually lower than the frequency of the incident photons. This process (inelastic scatter) is called the Raman effect. Raman scattering can be caused by a change in vibrational, rotational or electronic energy of a molecule. Chemists are considering primarily with the vibrational Raman effect, and the term Raman effect means vibrational Raman effect only in this text.

The Raman effect occurs when the incident photon interacts with the electric dipole of the molecule, and it is described in Figure 4. When an external field oscillating with a frequency ν_{ex} is applied to a molecule having a vibrational frequency ν_{vib} , the oscillation of an induced dipole moment p and the vibrational coordinate change q can be written respectively as

$$p = \alpha E = \alpha E_0 \cos(2\pi\nu_{ex}t) \quad \text{and} \quad q = q_0 \cos(2\pi\nu_{vib}t).$$

Since the molecular polarizability can be expanded as a Taylor series as a function of the vibrational coordinate as follows,

$$\alpha = \alpha_0 + \left(\frac{\partial \alpha}{\partial q}\right)_0 q + \frac{1}{2} \left(\frac{\partial^2 \alpha}{\partial q^2}\right)_0 q^2 + \dots$$

the induced dipole moment change in time can be approximated up to the first order as

$$p = \alpha_0 E_0 \cos(2\pi\nu_{ex}t) + \left(\frac{\partial \alpha}{\partial q}\right)_0 q_0 \frac{E_0}{2} \cos[2\pi(\nu_{ex} + \nu_{vib})t] + \left(\frac{\partial \alpha}{\partial q}\right)_0 q_0 \frac{E_0}{2} \cos[2\pi(\nu_{ex} - \nu_{vib})t].$$

Then, the oscillation modes of the dipole moment can be classified into the following three types:

$$(i) \quad \frac{\partial \alpha}{\partial q} = 0 \Rightarrow p = \alpha_0 E_0 \cos(2\pi \nu_{ex} t) \quad \text{Rayleigh Scattering}$$

$$(ii) \quad \frac{\partial \alpha}{\partial q} \neq 0 \Rightarrow \begin{cases} (\nu_{ex} - \nu_{vib}) & \text{Stokes Scattering} \\ (\nu_{ex} + \nu_{vib}) & \text{Anti-Stokes Scattering} \end{cases}$$

where are graphically described in Figure 4. The Rayleigh scattering is for an induced dipole oscillating with a frequency of ν_{ex} solely due to the external field, while the Stokes and anti-Stokes scattering are for the dipole oscillating with a frequency of $\nu_{ex} - \nu_{vib}$ and $\nu_{ex} + \nu_{vib}$ due to the coupling between the external field and its own vibrational mode, respectively.

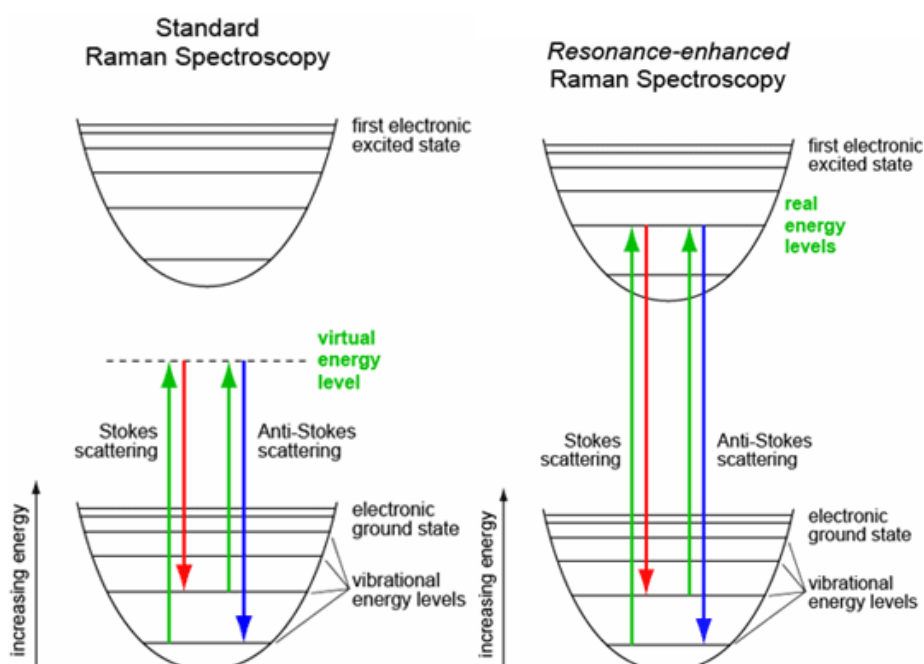


Figure 4 Energy level diagram for Raman scattering; Left: Standard Raman scattering, Right: Resonance-enhanced Raman scattering.

The energy difference between the incident and scattered photons is represented by the arrows of different lengths in Figure 4. The energy difference between the initial and final vibrational levels, or Raman shift in wavenumbers (cm^{-1}), is calculated through the equation below.

$$\tilde{\nu} = 1/\lambda(\text{incident}) - 1/\lambda(\text{scattered})$$

in which $\lambda(\text{incident})$ and $\lambda(\text{scattered})$ are the wavelengths (in cm) of the incident and Raman scattered photons, respectively.

The initial state in the majority of molecules is in the ground state because the thermal

population of vibrational excited states is low, even though not zero at room temperature. Therefore, the most scattered photon has lower energy and longer wavelength than the exciting photon (Stokes shift) in Figure 4. Additionally, a small fraction of molecules is in vibrational excited states following the Boltzmann population of states. Therefore, molecules in vibrational excited states go to the ground state and this scattered photon has a higher energy (anti-Stokes-shift) in Figure 4. The anti-stokes shifted Raman scattering is always weaker than the Stokes-shifted Raman scattering. These shifts in energy give information about the vibrational modes in the targeted molecule.

The gross selection rule for vibrational Raman transitions is that **the polarizability (α) should change as the molecule vibrates**. As homonuclear and heteronuclear diatomic molecules swell and contract during a vibration, the control of the nuclei over the electrons varies, and hence the molecular polarizability changes:

$$\frac{\partial \alpha}{\partial q} \neq 0$$

where q is the coordinate of the vibrational mode. Both types of diatomic molecule are therefore vibrationally Raman active. **The specific selection rule for vibrational Raman transitions in the harmonic approximation is $\Delta v = \pm 1$.**

The lines to high frequency of the incident radiation, the anti-Stokes lines, are those for which $\Delta v = -1$, as depicted in Figure 4. The lines to low frequency, the Stokes lines, correspond to $\Delta v = +1$. The intensities of the anti-Stokes and Stokes lines are governed largely by the Boltzmann populations of the vibrational states involved in the transition. It follows that anti-Stokes lines are usually weak, because very few molecules are in an excited vibrational state initially.

4. SERS (Surface-enhanced Raman Spectroscopy or Surface-enhanced Raman Scattering)

SERS is the abbreviation for “Surface-enhanced Raman Spectroscopy” or “Surface-enhanced Raman Scattering”. SERS is a very sensitive technique that enhances all molecular fingerprint of Raman with extremely high sensitivity. The SERS effect is generated when the targeted molecules are on nanostructured systems consisted of certain metals, such as gold or silver. When the electric field (light) at an incident wavelength where resonance with a plasmonic motion of metals occurs is given, the charge density oscillation is confined to metallic nanoparticles and it terms localized surface plasmon (LSPs). (Figure 5) Excitation of LSPs causes strong light scattering, intense surface plasmon absorption, and an enhancement of the local electromagnetic fields. On average, the enhancement factor by metallic nanostructured systems can be obtained in the ranges between 3 and 6 orders of magnitude. The intensity of Raman scattering is determined by the square of the electric field-induced dipole moment, P , where α is the molecular polarizability and E is the electric field incident upon the

molecule.²

$$P = \alpha \cdot E$$

From this equation, exceptionally increased Raman scattering is associated with the targeted molecules on the nanostructured sites such as the aggregation of AgNPs (hot spots), and chemical bond formation between the molecules and metal. The greater contribution to the overall SERS enhancement is attributed to the field enhancement mechanisms. The field enhancement mechanisms are caused by the amplification in the magnitude of both the incident and scattered electromagnetic (EM) fields resulting from the excitation of localized, coupled surface plasmons at hot spots. A smaller contribution to the overall SERS enhancement (maximum 3 orders of magnitude) is originated from the charge transfer (CT) enhancement mechanisms. This mechanism may be understood as follows; chemisorbed molecules on atomically clusters of silver atoms or through the nanosurface complexes scatter the incident light more efficiently than a free chemical species does.

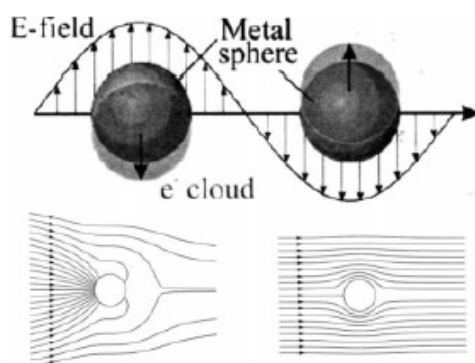


Figure 5. Top: Schematics for plasmon oscillation for a sphere, showing the displacement of conduction electron charge cloud relative to the nuclei. From *J. Phys. Chem. B* **2003**, *107*, 668. (copyright: 2003, the American Chemical Society. Bottom : Field lines of the pointing vector (excluding that scattered) around a small aluminum sphere illuminated by light of energy 8.8 eV where resonance occurs (left hand side) and 5. eV where there is no resonance (right hand side). From *Absorption and Scattering of Light by Small Particles*, Vol 1, John Wiley & Sons, Inc., New York. **1998**.

5. Comparison of Raman and IR Spectroscopy

Note that this section was adopted from the website: <http://www.chemvista.org/ramanIR4.html>

In a molecule with a center of symmetry it is seen that vibrations that are Raman active are IR inactive and vice-versa, this is called the Principle of mutual exclusion (eg, as in CO₂). In molecules with different elements of symmetry, certain bands may be active in IR, Raman, both or neither. For a complex molecule that has no symmetry except identity element, all of the normal modes are active in both IR and Raman. This does not; however mean that they can be observed. In both types the neighbouring strong bands may obscure weak bands, while

others may be intrinsically too weak to be observed even if they are theoretically “allowed”.

In general the strong bands in the IR spectrum of a compound correspond to weak bands in the Raman and vice versa. This complimentary nature is due to the electrical characteristic of the vibration. If a bond is strongly polarized, a small change in its length such as that occurs during a vibration, will have only a small additional effect on polarization. Vibrations involving polar bonds (C-O, N-O, O-H) are therefore, comparatively weak Raman scatterers. Such polarized bonds, however, carry their charges during the vibrational motion, (unless neutralized by symmetry factors), which results in a large net dipole moment change and produce strong IR absorption band. Conversely, relatively neutral bonds (C-C , C-H, C=C) suffer large changes in polarizability during a vibration, though this is less easy to visualize. But the dipole moment is not similarly affected and vibrations that predominantly involve this type of bond are strong Raman scatterers but weak in the IR.

In molecules having inversion center, none of the normal modes of vibrations will be both Raman and IR active. This is known as “mutual exclusion principle”. A simple molecule which obeys this principle is CO₂. Carbondioxide has an inversion center or center of symmetry. The following are its normal modes of vibrations. The IR and Raman active modes are indicated below each type of vibration.

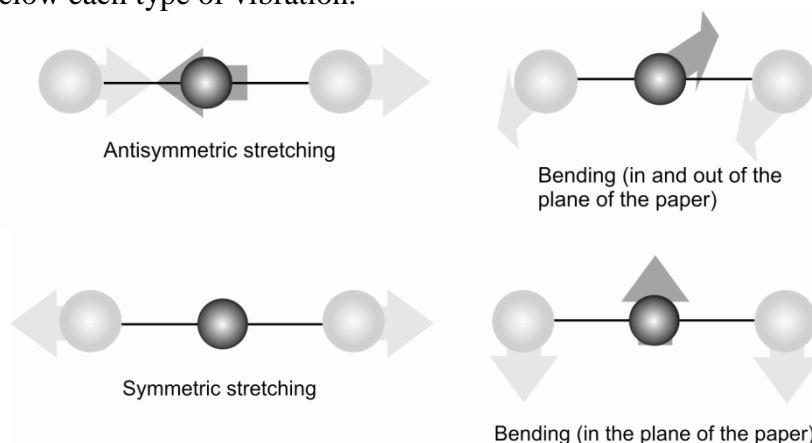


Figure 6. The upper two vibrational modes are IR active since they change the dipole moment of CO₂, whereas the lower two are Raman active because they change the polarizability without altering the dipole moment.

Raman	IR
It is due to the scattering of light by the vibrating molecules.	It is the result of absorption of light by vibrating molecules.
The vibration is Raman active if it causes a change in polarizability.	The vibration is IR active if there is a change in dipole moment during the vibration.
The molecule need not possess a permanent dipole moment.	The vibration concerned should have a change in dipole moment due to that

	vibration.
Water can be used as a solvent.	Water cannot be used due to its intense absorption.
Sample preparation is not very elaborate sample can be almost in any state.	Sample preparation is elaborate Gaseous samples can rarely be used.
Gives an indication of covalent character in the molecule.	Gives an indication of ionic character in the molecule.
Cost of instrumentation is very high	Comparatively inexpensive.

Table 1. Comparison of Raman and IR Spectroscopy

6. Colloidal Silver Nanoparticles (AgNPs) Synthesis

In this laboratory experiment, the colloidal AgNPs are synthesized by the reduction of silver nitrate (AgNO_3) with sodium borohydride (NaBH_4). This method slightly modified Creighton method,³ which has been widely used due to its simplicity, accessibility, relatively low cost and time efficiency. The AgNO_3 : NaBH_4 molar ratio of 1:2 was optimized to give a stable final colloid and to ensure complete reduction of Ag^+ during minimizing excess NaBH_4 . Additionally, each solution should be used ice-cold highly-purified water and kept cold to avoid the hydrolysis side reaction of NaBH_4 . The reaction should be performed in an open vessel to prevent the buildup of H_2 gas and possible explosions.

The proposed mechanism for the formation of AgNP seeds and growth is summarized in Figure 7.

(1) step : NaBH_4 donates a hydride to Ag^+ to form silver hydride (AgH), borane (BH_3), and sodium nitrate (NaNO_3).

(2) step : the nucleation of the AgNP seed initiated with the dimerization of AgH to form Ag_2 and H_2 .

(3) step : the seed growth is maintained by Ag_2 forming a complex with Ag^+ from AgNO_3 and subsequent reduction with NaBH_4 .

(4) step : the AgNP size increases when two larger seeds, $\text{Ag}(\text{Ag})_n\text{AgH}$, dimerize with H_2 as a bi-product.

(5) step : the stepwise growth process continues leading to the formation of stabilized, colloidal AgNPs surrounded by $\text{B}(\text{OH})_3$ and NaNO_3 ionic compounds.

(7-8) step : the excess of NaBH_4 and BH_3 will cause hydrolysis to boronic acid ($\text{B}(\text{OH})_3$) as described in Figure 8 and remain in aqueous solution with a small amount of NaOH as by-product.

(6-11) step : the formation of H_2 gas will be bubbled from the reaction mixture.

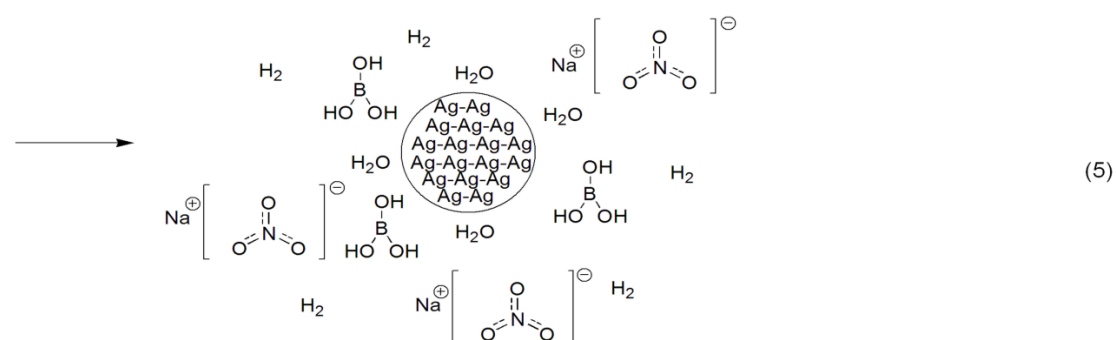
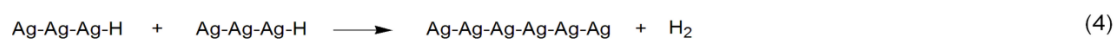
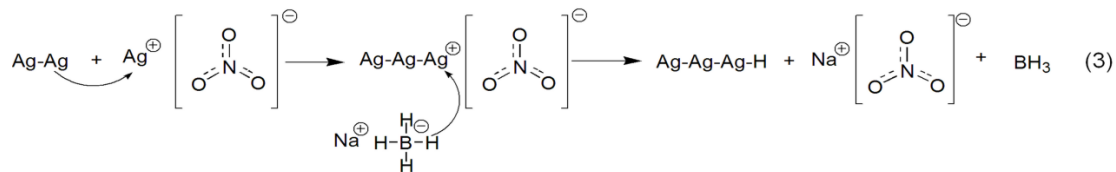
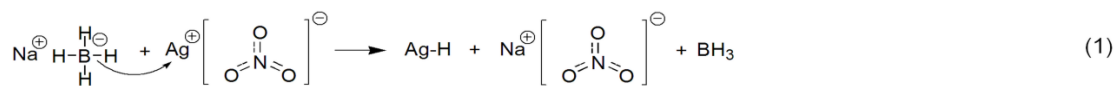


Figure 7. Schematic of the five steps mechanism proposed for the formation of colloidal silver nanoparticles in the Creighton method.

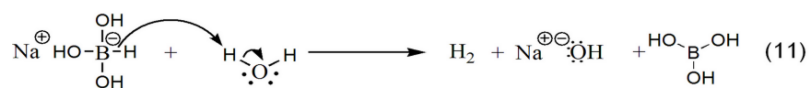
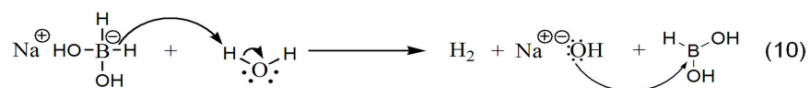
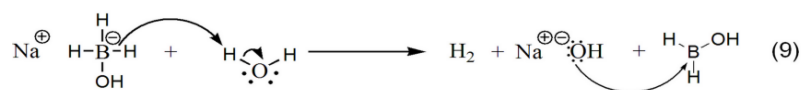
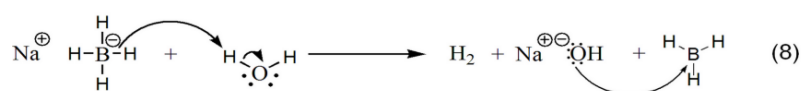
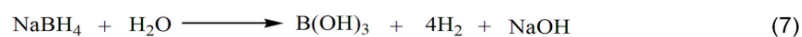
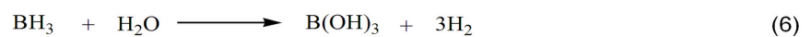


Figure 8. Hydrolysis of borane and sodium borohydride

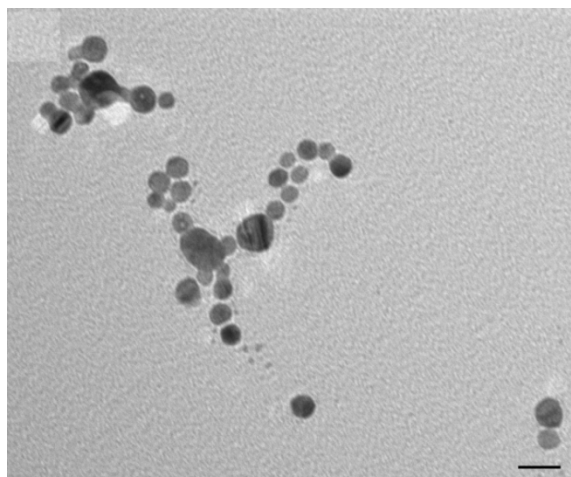


Figure 9. TEM image of the Creighton colloid showing the silver nanoparticles size, shape, aggregation state, and distribution. The scale bar is 20 nm. The image was taken from Ref. 1.

Figure 9 shows a TEM image of the silver nanoparticles synthesized along the experimental procedure described above.

7. Optical properties of AgNPs

The anomalous electron configuration of silver metal is $[\text{Kr}]5s1^4d^{10}$, and as a result, the lone s electrons in the atoms of AgNPs are collectively displaced on the surface of AgNPs when an electrical field at the frequency of the laser light is employed. The attraction between the positive nuclei and the negatively charged electron cloud develop a restoring electrostatic force. The Coulombic attraction arises the collective oscillation of the electron cloud in the nuclear framework. This collective oscillation of the electrons is dipole surface plasmon resonance (SPR). The SPR of AgNPs typically generates an absorption maximum at 400 nm and represents the yellow color of the colloid (figure 10). The SPR peak profile informs the properties of AgNPs such as the amount, shape, size, and aggregation.

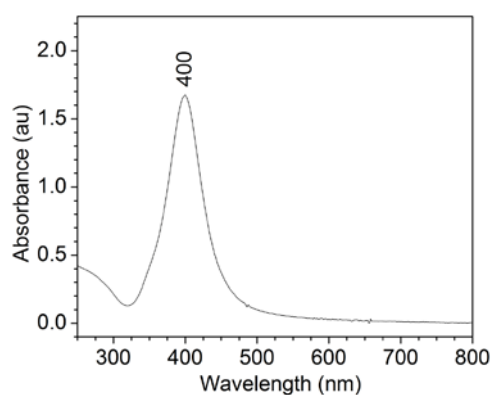


Figure 10. UV-Visible absorption spectrum of Creighton silver colloid.

8. Rhodamin 6G (R6G) and SERS samples preparation

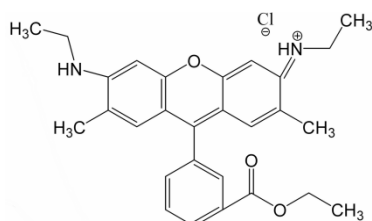


Figure 11. Chemical structure of rhodamine 6G

Rhodamin 6G (R6G) is a fluorescent dye, generally used as a target molecule in SERS for testing the enhancement efficiency of SERS nanosubstrates (figure 11). In Figure 12, Raman spectrum was obtained from gold substrates. The Raman peaks observed at 1180 cm⁻¹ indicate C-C stretching vibrations, and signals at 1308, 1360, 1506, and 1648 cm⁻¹ are due to the aromatic C-C stretching vibrations.⁴

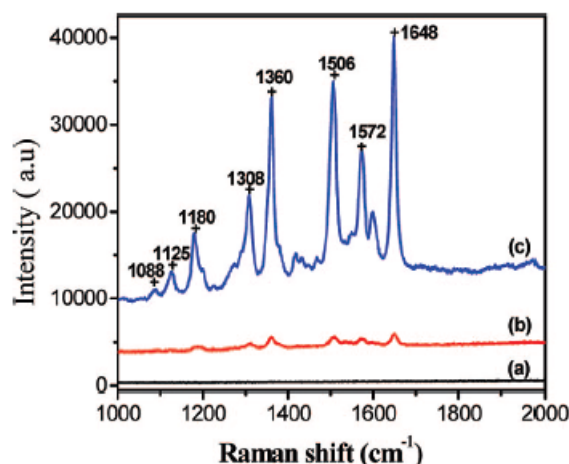


Figure 12. Raman spectra of (a) 100mM rhodamine 6G (R6G) adsorbed on quartz, (b) 0.1 μ M R6G adsorbed on a gold nanosphere-modified quartz substrate, and (c) 5.0 nM R6G adsorbed on a gold nanochainmodified quartz substrate.

In this experiment, the SERS samples are prepared by 900 μ L of the 2.22×10^{-6} M R6G solution and 100 μ L of the KBr solution to 1 mL of colloid. In the final SERS samples, the concentrations of R6G and KBr will be approximately 1.0×10^{-6} M and 5.0×10^{-2} M, respectively. The KBr salt solution plays the role to enhance the formation of AgNP-aggregates (i.e., hot spots) and to increase the SERS enhancement factors. The Br⁻ ions surrounding the AgNP surface generate strong electrostatic bridges with the R6G cations. In addition, a small amount of halide ions raises the reproducibility of the SERS spectra.

9. Surface enhancement factors

The surface enhancement factor for AgNPs is calculated from the given equations:

$$EF = \frac{I_{sers}/C_{sers}}{I_{ref}/C_{ref}} \times \frac{acqtime_{ref}}{acqtime_{sers}}$$

where I_{sers} and I_{ref} represent the intensities of the highest band (in Figure 11, 1648 cm^{-1}) for the R6G adsorbed on the AgNPs substrate and the normal Raman of H_2O , whereas C_{sers} and C_{ref} represent the corresponding concentrations of R6G on these samples. It indicates how much more the Raman signal can be enhanced from SERS in compared with the normal Raman.⁴

10. IR spectroscopy and sample preparation of R6G

FT/IR spectra of R6G solution demonstrate a band at 1716 cm^{-1} which corresponds to the C=O vibration in ester group, and the bands at 1650 cm^{-1} and 3370 cm^{-1} indicating to the N-H stretching vibration and scissor bending vibration of the amine groups (Figure 13). The peaks at 1452 cm^{-1} , 1500 cm^{-1} , 1566 cm^{-1} , and 1609 cm^{-1} indicate the vibration of aromatic ring skeleton, and the peaks at 1370 cm^{-1} and 2985 cm^{-1} are occurred by C-H stretching vibration and bending vibration of methyl, respectively. In addition, 1312 cm^{-1} and 1532 cm^{-1} peaks are the in-plane bending vibration and stretching vibration of C-N group, respectively.

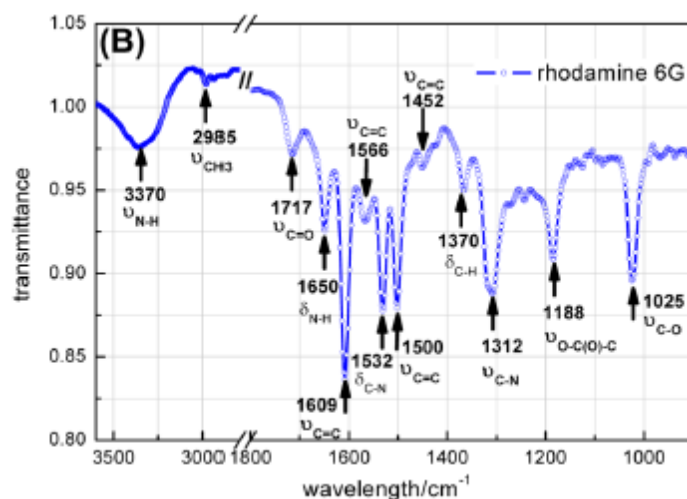


Figure 13. FTIR spectra of R6G. This image has been taken from Ref. 5.

11. Equipment

This will be replaced by the introductory lecture on the FT-IR/Raman Spectroscopy and related notes.

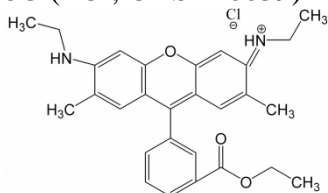
Pre-Laboratory Data Evaluation

1. What is the Raman scattering effect? And explain its limitation
2. What does SERS stand for?
3. Explain the two mechanisms accounting for the huge enhancement observed in SERS experiments in comparison with the normal Raman spectroscopy.
4. How do the colloidal silver nanoparticles affect in SERS and how can they be synthesized?
5. What is the difference between the Raman spectroscopy and IR spectroscopy?

Materials

Reagents

- Silver nitrate (Sigma-Aldrich Co., Llc, CAS #209139)
- Sodium borohydride (Sigma-Aldrich Co., Llc, CAS#452882)
- Rhodamine 6G (TCI, CAS#R0039)



- Potassium bromide (Sigma-Aldrich Co., Llc, CAS#243418)
- Methanol (JUNSEI, CAS#73125-1280)

Apparatus

- Infrared (IR) spectroscopy
- Raman spectroscopy

Safety and Hazards

- Silver nitrate, sodium hydroxide, and hydrochloric acid : corrosive materials that cause burns upon contact with the skin and eyes.
- Sodium borohydride : flammable and toxic
- Rhodamine 6G and potassium bromide : toxic and may cause eye, skin, or respiratory track irritations.

Equipment

- Raman spectrometer
- FT/IR spectrometer

Experimental Procedure

Synthesis of Silver nanoparticles (AgNPs)

1. A 50-ml vial is covered in aluminum foil, and is placed in an ice bath on a stir plate.
2. A 30 ml aqueous solution of NaBH₄ solution (2.0 mM) is prepared with de-ionized water in the 50-ml vial in 1 step.
3. A 5 ml aqueous solution of AgNO₃ solution (1.0 mM) is dissolve with de-ionized water in a vial covered in aluminum foil.
4. While moderately stirring the NaBH₄ solution, 5 ml of AgNO₃ solution is added drop wise slowly from a burette at a rate of about one drop per second.
5. While AgNO₃ solution is being added, the solution starts turning color to light yellow.
6. Stirring is maintained for another 45 to 60 min in the ice bath, and the final solution should be clear and intensely yellow.
7. This AgNPs solution is stable for approximately 5-6 months when kept in a cool, dark environment.

Preparing Rhodamin 6G (R6G) and SERS sample

1. R6G (1.0×10^{-3} M, 2.22×10^{-5} M, 1×10^{-5} M) and KBr (1.0 M) aqueous solutions are prepared.
 2. The SERS sample is prepared by adding 900 μ l of 2.22×10^{-5} M R6G solution and 100 μ l of 1.0 M KBr solution to 1.0 ml of AgNPs solution. (the final concentrations of R6G and KBr in the SERS sample are 1.0×10^{-5} M, and 5.0×10^{-2} M.)
- ** The KBr solution affects to promote the aggregation of AgNPs, and the aggregated AgNPs improve the SERS enhance factors. The Br⁻ ions surround the AgNP surface and lead to form strong electrostatic bridges with R6G cations.

Measurement of Raman and surface-enhanced Raman spectroscopy (SERS)

1. The normal Raman and SERS measurements are performed by using a He-Ne laser with an excitation wavelength of 632.8 nm and power of 17 mW at the sample.
2. The normal Raman spectrum and the SERS sample are obtained from the 1.0×10^{-3} M R6G solution, the 1.0×10^{-5} M R6G solution, and the SERS sample within a capillary tube.
2. The acquisition times are set at 30 s for normal Raman measurements, and 3 s for SERS spectra. Each normal Raman and SERS spectral window is averaged over 5 cycles for a good signal to noise ratio.

How to operate Raman spectroscopy

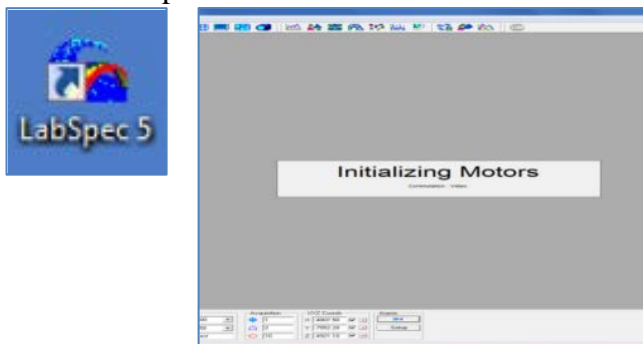
1. Hold the filter for 632.8 nm laser in the right place, and make the path of 632.8 nm laser by raising the filter as shown below



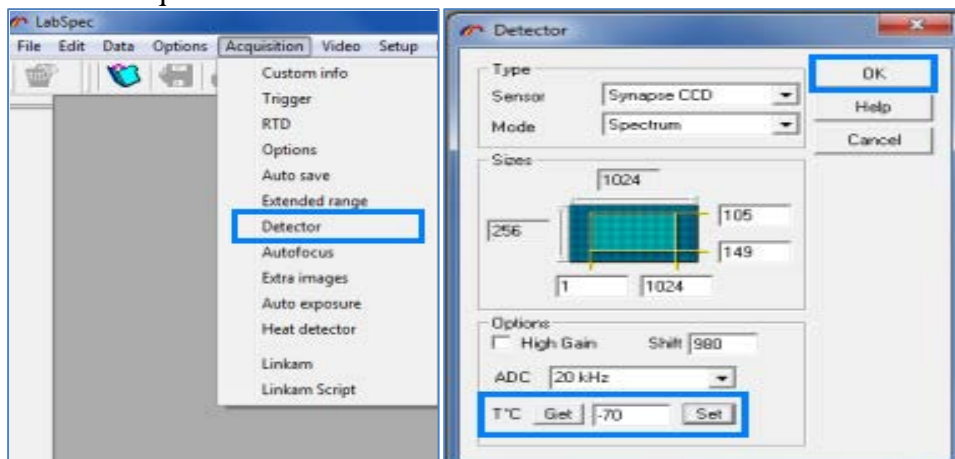
2. Push the power button on the main box, and turn on the 632.8 nm laser on.



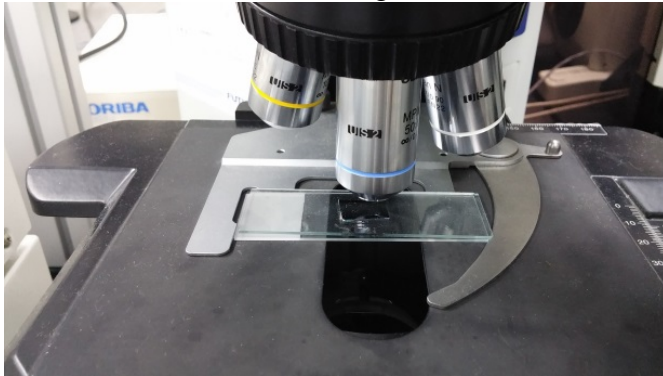
3. Run "LabSpec 5"



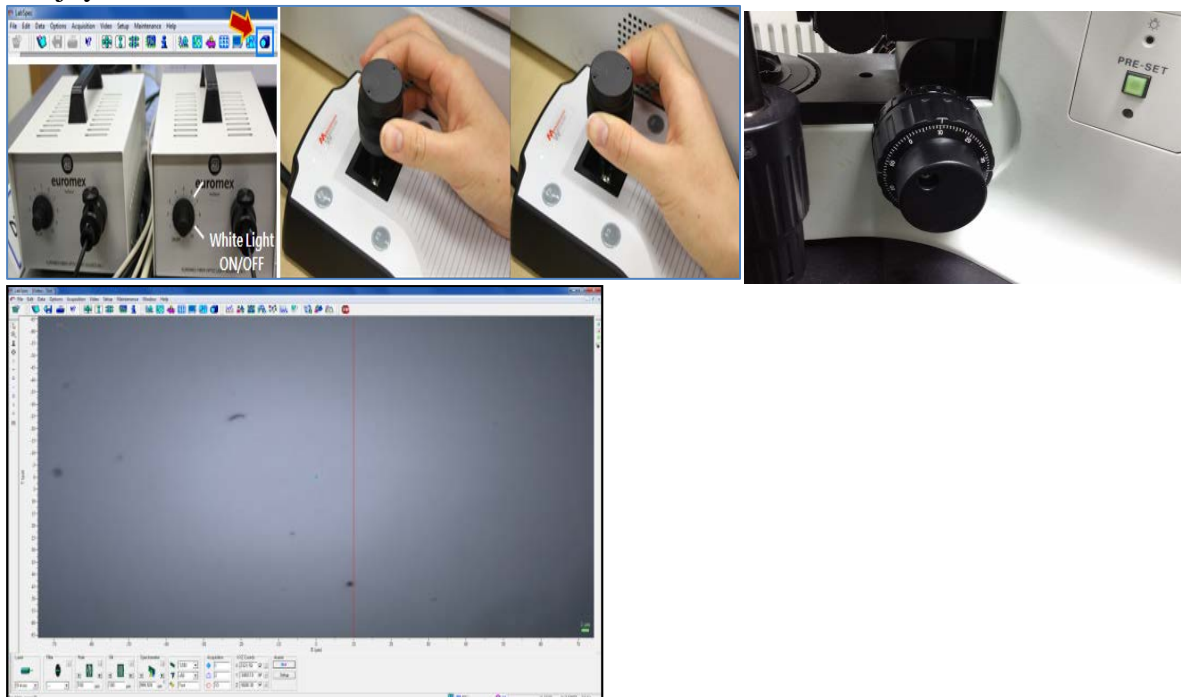
4. Menu>Acquisition>Detector : set -70 °C and OK.



5. Put the Si-wafer on the stage. (the Si-wafer is used as the reference for calibration)



6. Lab Spec Camera on >White light on>focusing by using lever beside the stage (x,y,z-axis), and joy stick.



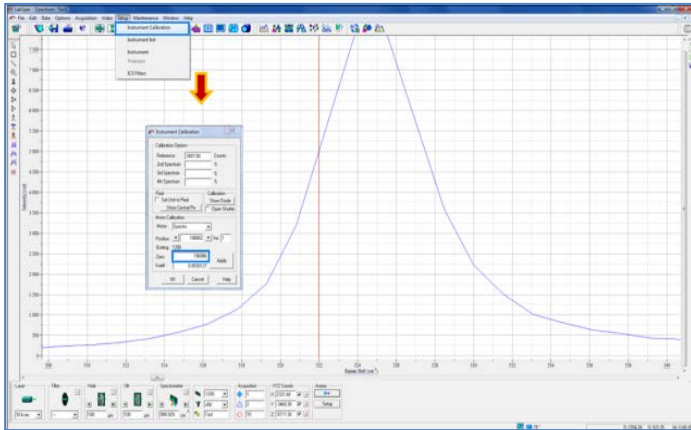
(the focused silicon sample)

7. After focusing, turn off the white light, and turn on the laser.



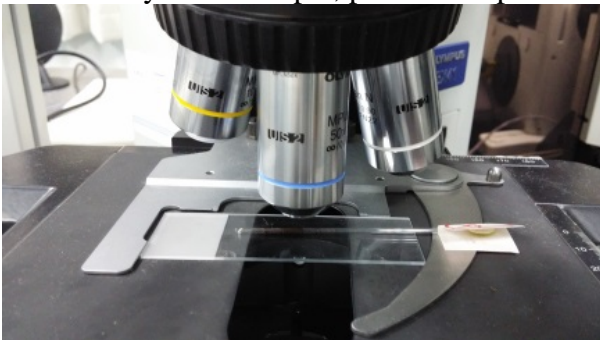
8. Click “RTD Acquisition Mode ” and get a real time spectrum.

9. Zoom in the center of peak around 521 cm^{-1} , Click Menu>Setup>Instrument Calibration.



10. By changing the value of zero, set the center value of spectrum at 521 cm^{-1} .

11. To analyze the sample, put the sample on the stage.

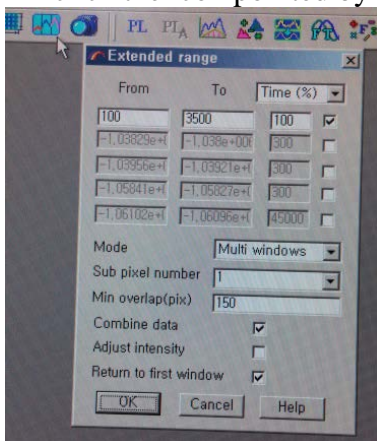


12. Set the condition of estimation as shown below.



13. Set the range of wavelength for Raman spectrum at “Extended range”

**click the icon pointed by cursor



14. After focusing the sample like following the described order before, get the Raman

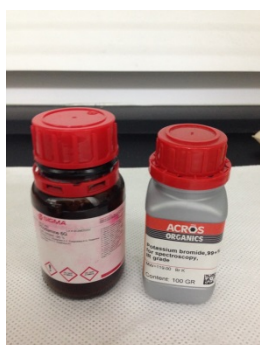
spectrum of the sample by clicking the icon.



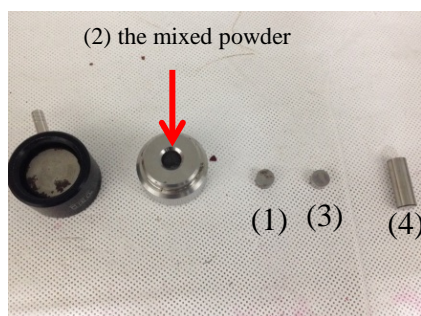
15. After finishing the analysis, turn off the laser and send the data by e-mail, not USB.

Measurement of IR spectroscopy

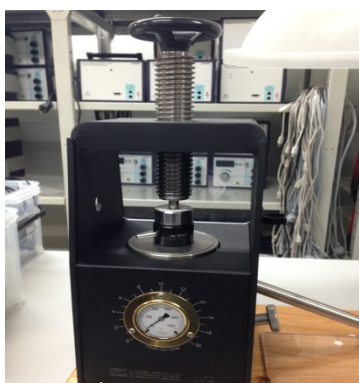
A. Preparation of R6G for IR measurement.



1. Mix R6G and KBr powder to pelletize them (the powder should be homogeneously mixed)



2. Assemble the components following the numbering (Put the mixed powder in the place, is described in right picture with arrow)



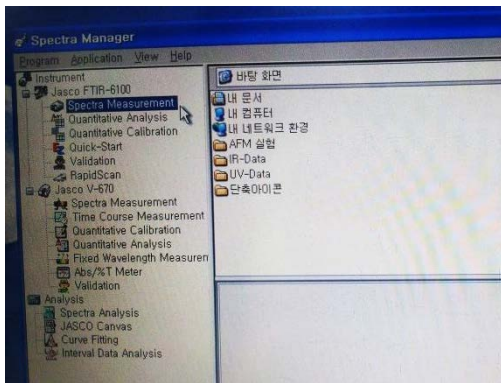
3. Set the holder as shown in the left picture and compress the powder with the instrument

4. Place the pelletized sample on the IR holder

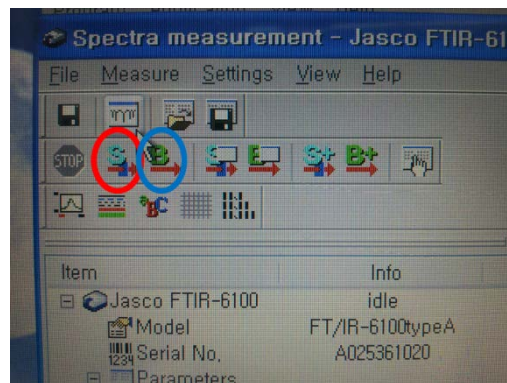
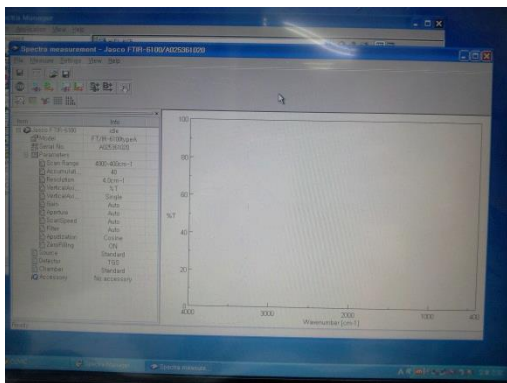
B. measurement of FT/IR spectroscopy



1. Turn on the power switch of Jasco FT/IR (the power switch is circled with red line in Figure B1).
3. Click the icon “SpectraManager” which is the program for spectra measurement.
4. Click “Spectra Measurement” to initialize the FT/IR spectroscopy.



5. Click “Spectra Measurement” (Figure B2)
6. Mount the sample for baseline. (Figure B3)
7. On the window of “Spectra Measurement-Jasco FTIR-6100”, click “B→” which is circled with the blue line to measure the baseline in FT/IR spectroscopy. (Figure B4,5)



8. Mount the sample of R6G and measure the FT/IR spectroscopy by clicking the icon “S→” which is circled with the red line. (Figure B5)

Post-Laboratory Data Evaluation

1. Plot, preferably in one graph, the normal Raman spectrum of the colloid alone, the normal Raman spectrum of the 1.0×10^{-1} M R6G solution, and the SERS spectrum of 1.0×10^{-6} M R6G adsorbed on AgNP surface with the peaks of interest labeled.
2. Which is the most intensive peak band? What value were obtained for I_{sers} and I_{ref} represent the intensities of the 1648 cm^{-1} band and the most intensive peak band for the R6G adsorbed on the AgNPs substrate and the normal Raman of H_2O ?
3. Obtain the surface enhance factor.
4. Plot the IR spectrum of the R6G solution and label the significant peaks.
5. Compare the IR spectrum and Raman spectrum, and discuss about the difference between them according to the selection rules of IR/Raman spectroscopy.

References

1. "Estimating the analytical and surface enhancement factors in surface-enhanced Raman Scattering (SERS): a novel physical chemistry and nanotechnology laboratory experiment" *Journal of chemical education*, 89 (2012), 286-290.
2. "Surface-enhanced Raman spectroscopy", *Analytical Chemistry*, 61 (1989) 401A-411A
3. "Plasma resonance enhancement of Raman scattering by pyridine adsorbed on silver or gold sol particles of size comparable to the excitation wavelength", *Journal of the Chemical Society, Faraday Transactions 2: Molecular and Chemical Physics*, 75 (1979) 85-94.
4. "Water-soluble conjugated polymer-induced self-assembly of gold nanoparticles and its application to SERS" *Langmuir*, 24 (2008), 10608-10611.
5. "Competitive adsorption of dopamine rhodamine 6G on the surface of graphene oxide" *ACS Applied materials & interface*, 6 (2014) 2459-2470.
6. *Atkins' Physical Chemistry* / Peter Atkins / Oxford university press (8th edition).
7. Donald A. McQuarrie and John D. Simon, "Physical Chemistry: A Molecular Approach", University Science Books, 1997