Structural Biology
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Lecture – 34
Infrared and Raman Spectroscopy for Protein

Hi everyone, welcome again to the course of structural biology. As you already know that we are going through structural biology techniques and currently we are in the module of spectroscopy. Today we have already discussed about the basic of spectroscopy CD, UV, fluorescence's. Today we are going to talk about another technique which are very important in protein. People have not used it a lot but it is used to get fingerprints.

And I am talking about IR spectroscopy also I would discuss about another one which is Raman because you always know IR and Raman comes together and I will talk in the later class how Raman have a very important role in determining protein structure.

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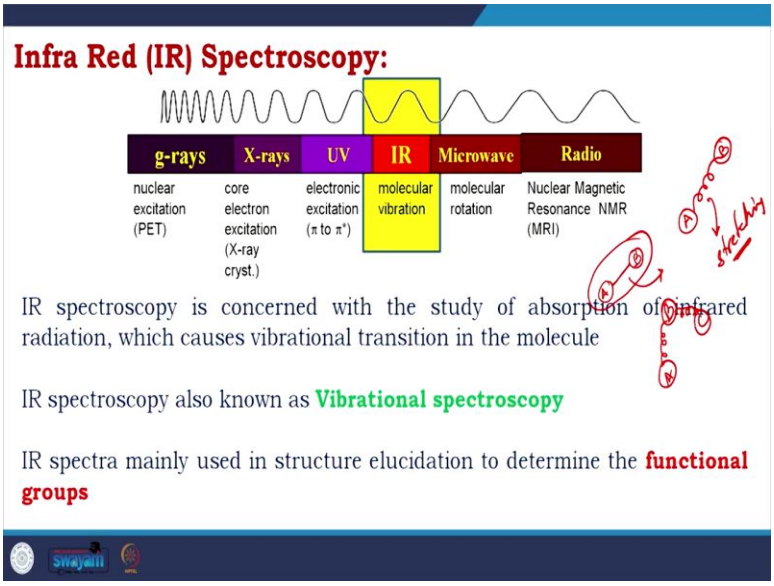

So, let us starts with infrared spectroscopy as you know the electromagnetic spectrum and infrared is in between UV and microwave. Infrared is generally influenced by vibration when I say vibration I mean bending and stretching. So, let us think about you have molecule or atom A and B connected through a bond. As we have discussed we now know that these binding could be explained in terms of connectivity as a spring.

So, now it would show stretching, is this stretching is characteristic if it is characteristic to anything then it would help us to characterize a particular molecule solvent functional group. Again if you have 3 atoms again you connect them with spring and you look at the bending. So, that is where infrared or IR spectroscopy works. So, IR spectroscopy is concerned with the study of absorption of infrared radiation which causes vibrational transition in the molecule.

IR spectroscopy also known as vibrational spectroscopy because it deals with the; vibrational transitions which we have discussed here, IR spectra mainly used in structure elucidation to determine the functional groups. As I told you are getting a particular bending or stretching if it is characteristic to some molecule then you get the identification there. So, you get particular spectra of that functional group.

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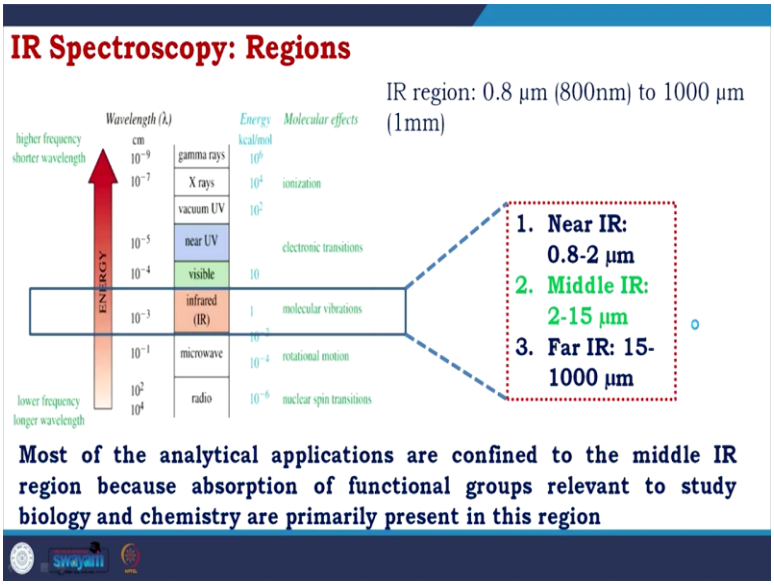

Now you see the position of the infrared. The infrared is 0.8 micrometer to 1000 micrometer wavelength. It could be divided into3 distinct part one is near infrared which is 0.8 to 2 micrometer. Middle infrared which is 2 to 15 micrometer and for infrared which is 15 to 1000 micrometer. Most of the analytical applications are confined in the middle IR region because absorption of functional groups relevant to study biology and chemistry are primarily present in this region.

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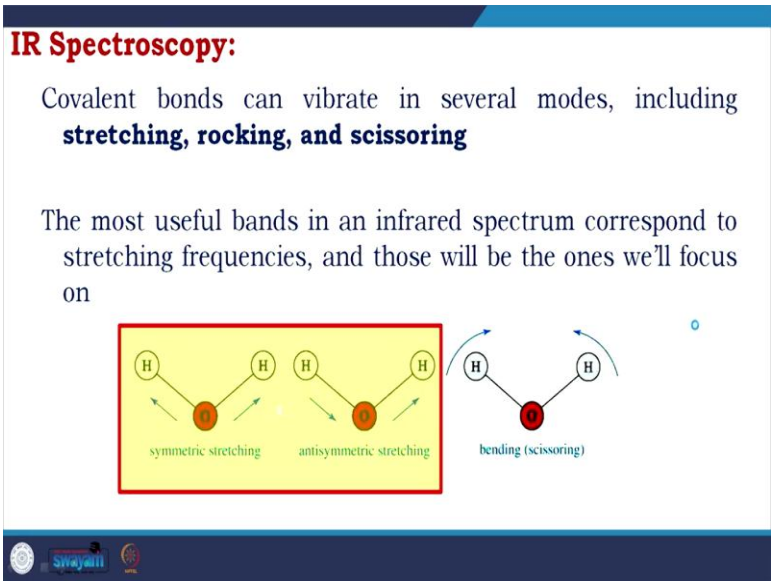

As I told the covalent bond can vibrate in several modes including stretching, rocking, scissoring if you see symmetric stretching antisymmetric or asymmetric stretching and bending or scissoring. So, the most useful bands in an infrared spectrum correspond to the stretching frequencies and those will be the ones we will focuses on which are the ones? These are the ones symmetric and asymmetric stretching.

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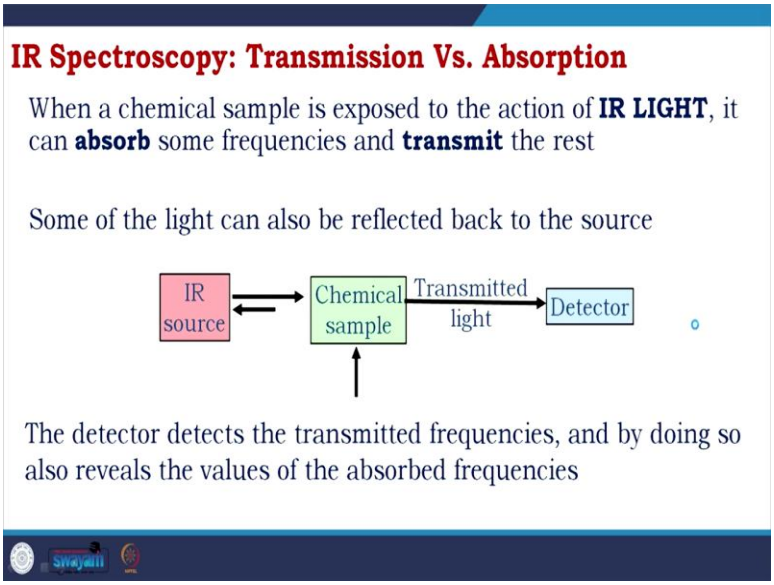

Transmission versus absorption this is you know everywhere we go in spectroscopy we look at them when a chemical sample is exposed to the action of infrared light it can absorb some frequency and transmit the rest. Some of the light can also be reflected back to the source we

have talked about interaction of matter with any light source and absorption, scattering, transmission, reflection all are possibility.

So, if you look at you have a IR source it go to the chemical sample of the sample which you put under experimental condition then the transmitted light goes to the detector and detection happen. The detector detects the transmitted frequencies and by doing. So, also reveals the values of the absorbed frequency.

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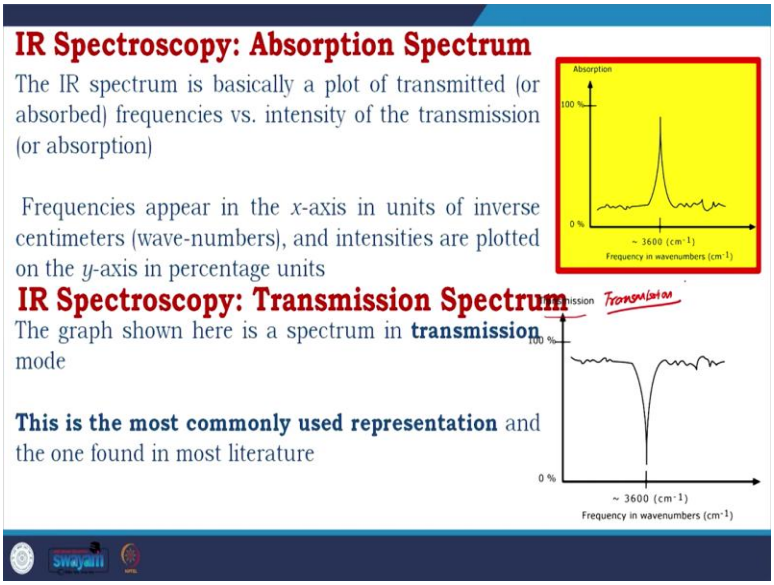

The absorption spectrum the IR spectrum which we see is basically a plot of transmitted or absorbed frequencies versus intensity of the transmission or absorption. So, if you see here absorption and frequency absorption is percentage frequency is in wave numbers centimeter inverse. Frequencies appear in x axis in unit of inverse centimeters wave numbers and the intensities are plotted on the y axis in percentage units.

This is transmission this is overlapped sorry. So, this is transmission the graph shown here is the spectrum in transmission mode. So, 2 modes you will see absorption spectrum absorption mode transmission spectrum in transmission mode. This is the most commonly used representation and one found in the literature. So, while we will go through the literature you will see these types of spectrum are common. We will use this in our representation also.

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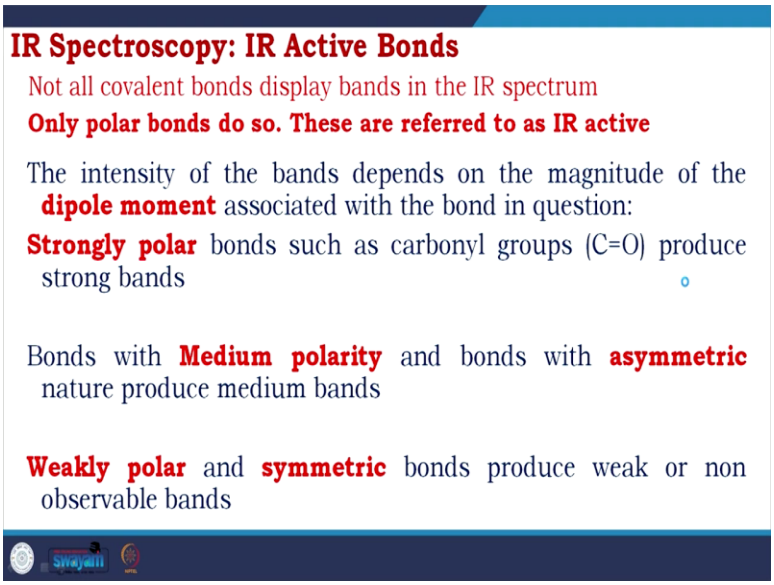

IR spectroscopy band IR bands can be classified as strong. If you see such a long band you will see strong medium like this and weak like this depending on their relative intensities in the infrared spectrum. IR active bonds not all covalent bonds display bands in the IR spectrum only polar bonds do so. These are referred to as IR active. The intensity of the bands depends on the magnitude of the dipole moment associated with the bond in question.

Strongly polar bonds such as carbonyl groups produce strong bands. Bonds with medium polarity and bonds with asymmetric nature produce medium bands. Weakly polar and symmetric bonds produce weak or non observable bands. So, strong band, medium band, weak band, no band these are the categorization that would happen depending on the dipole moment associated with the bond.

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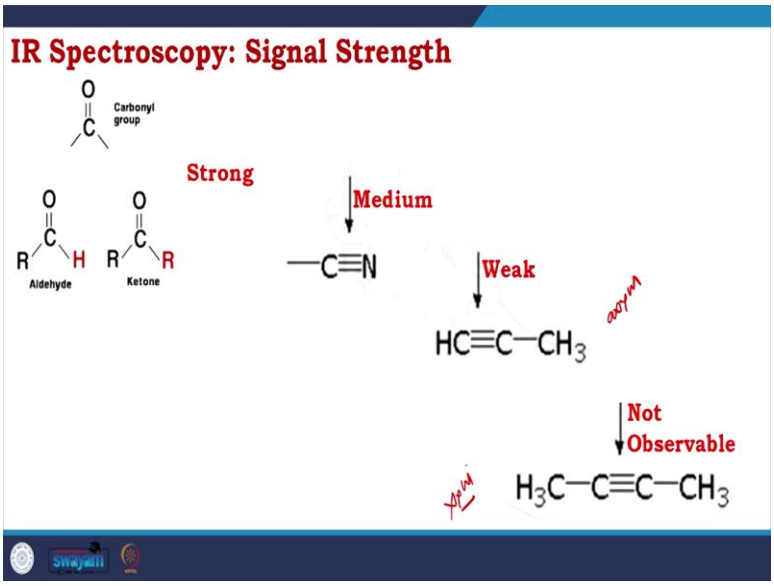

Signal strength to the molecule if you look at carbonyl group aldehyde and ketone these are strong. If you look at C triple bond N they are medium, if you look at C triple bond C they are weak and if you look at C triple bond C with methyl group in both the sides. So, giving the. So, this is asymmetric, this is symmetric. So, there is not observable or no band in IR spectroscopy not any one, we will talk about it.

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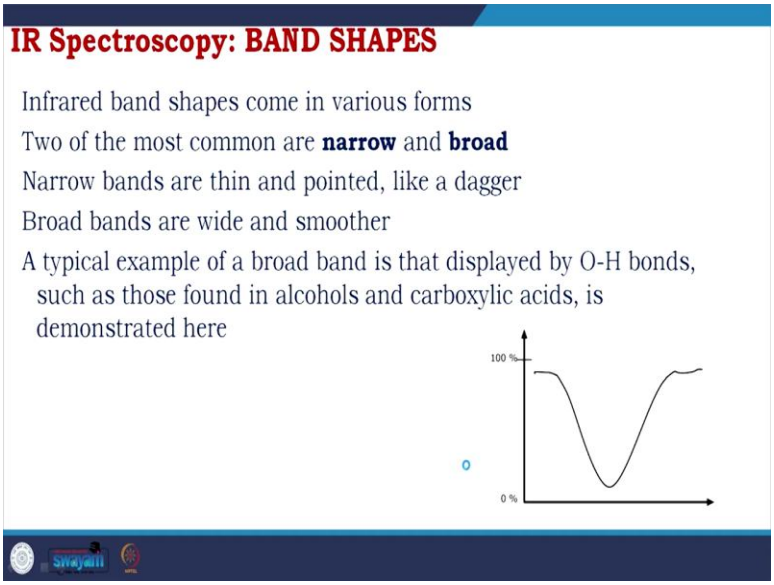

IR spectroscopy band shapes infrared band steps come in various forms 2 of the most common are narrow and broad. Narrow bands are thin and pointed like a dagger we will see them later broad bands are wide and smoother. A typical example of broadband is displayed by hydroxyl

bonds such as those found in alcohol and carboxylic acid we will see there see here it is the hydroxyl group.

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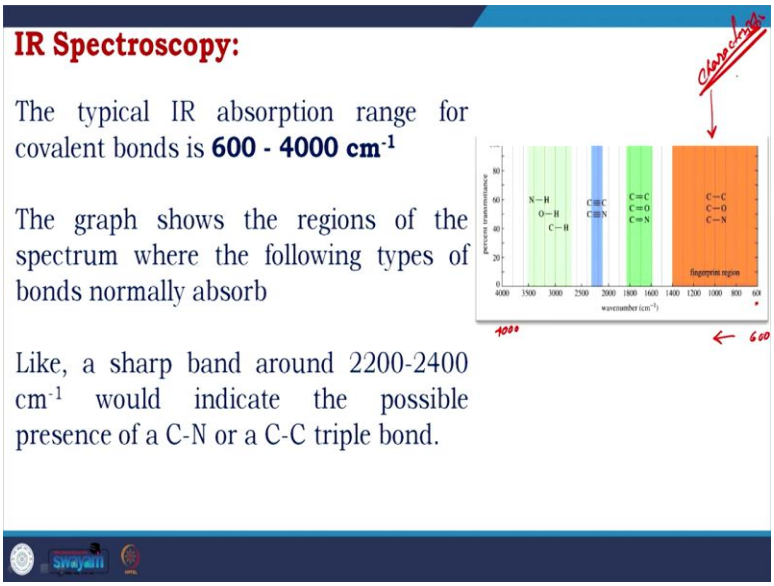

IR spectroscopy for different molecules a typical IR spectrum range for covalent bond is 600 to 4000 centimeter inverse. If you see here it is 600 to 4000 that is the range. The graph shows the region of the spectrum where the following type of bonds normally absorb. If you see NH OH CH they absorb within 3500 to 3000, 2500 to 2000 it is C triple bond C and C triple bond N double bonds are within 1800 to 1600 and C single bonds are actually characteristic that is why it is they are characteristic.

Characteristic means they do not change much. So, that is why if you get around this you know these are these bonds. Like a serve band around 2200 to 2400 centimeter inverse would indicate the possible presence of a C N or C C triple bond.

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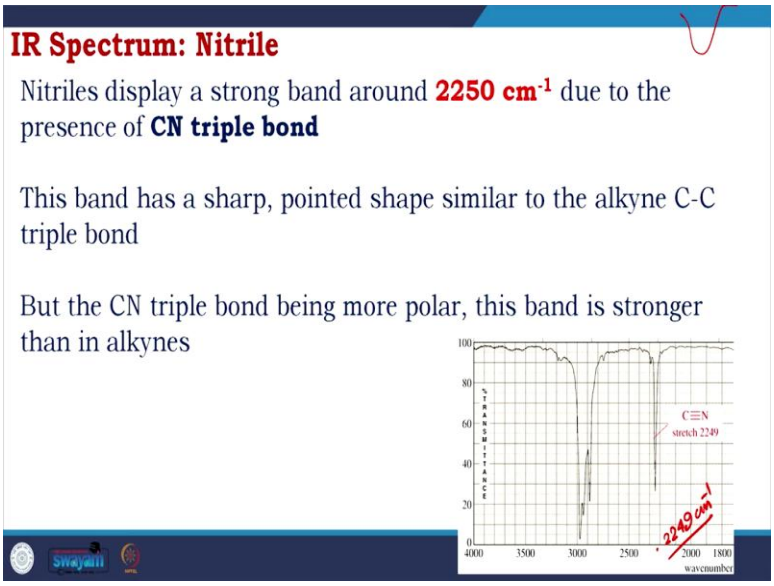

So, coming to different one now you could see these are thin ones we will talk about broad ones which we have shown already that would be like hydroxyl we have seen. Nitrides display strong band around 2250 centimeter inverse due to the presence of C N triple bond. So, here it is around 2249 centimeter inverse this band has a sharp pointed shape similar to the alkyne C triple bond C actually. But the C N triple bond being more polar this band is stronger than the alkynes.

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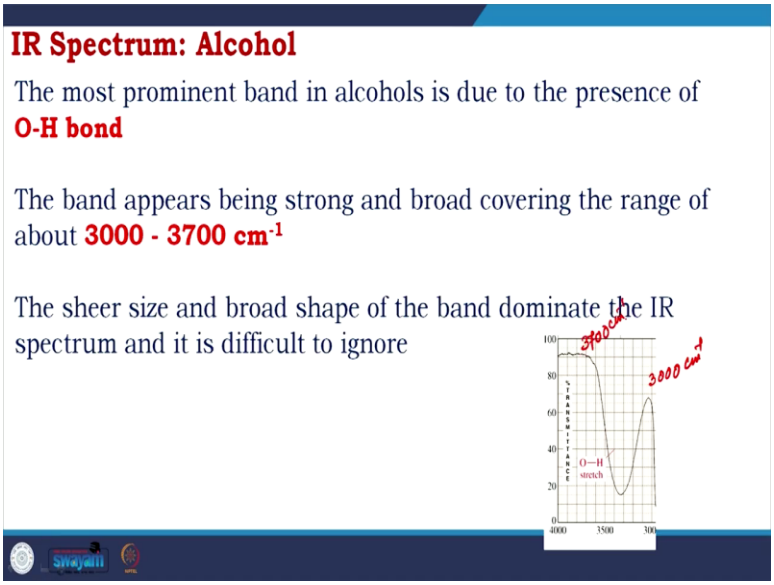

Alcohol: The most prominent band in alcohol is due to the presence of O-H or hydroxyl group the band appears being strong and broad covering a range of about here if you see it is started around 3000 centimeter inverse and it goes around 3700 centimeter inverse. The shear size and

broad shape of the band dominate the IR spectrum and it is difficult to ignore. So, what it means if you have a hydroxyl group you will get a band that is a surety.

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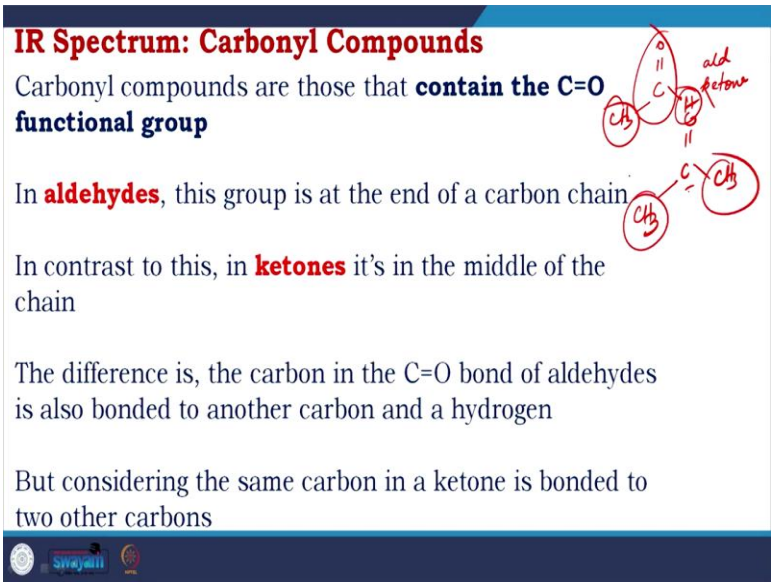

So, carbonyl compounds: The carbonyl compounds are those that contain the C double bond O functional groups among them we know that there are this is the carbonyl group. Now you have CHO aldehyde and ketone shear. In aldehyde this group is at the end of carbon chain because after that it is hydrogen. In contrast to this in ketone it is in the middle of the chain because. So, it is middle the difference is the carbon in the C double bond O bond of aldehyde is also bonded to another carbon and a hydrogen but considering the same carbon in a ketone is bonded to 2 other carbons. So, if you think here CH3 CHO one carbon one hydrogen CH3 CO CH3 2 carbon.

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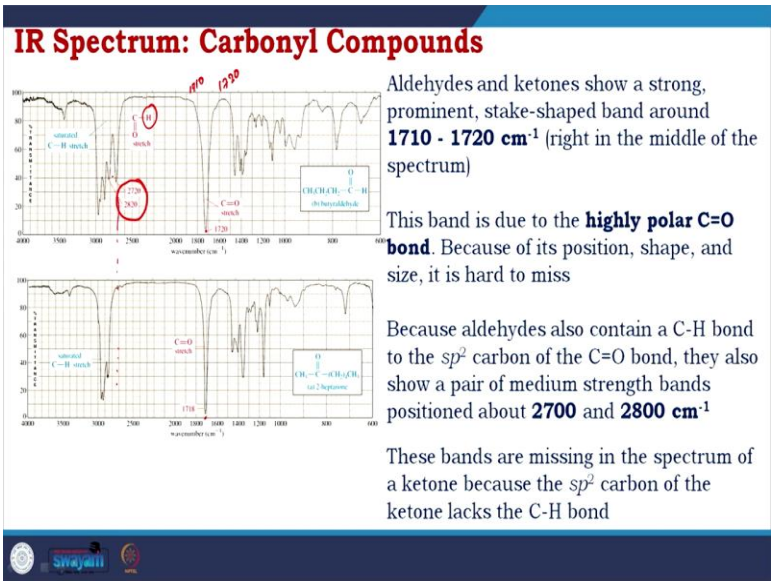

So, aldehydes and ketones show a strong prominence takes shape band arounds 1710 to 2720 centimeter inverse which is here you will see here you get a band 1710 to 1720. This band is due to the highly polar C double bond O bond because of its position shape and size it is hard to miss again. Because aldehydes also contain a C-H bond to the Sp 2 carbon of the carbonyl bond they also show a pair of medium strength bands position about 2700 and 2800. So, when you are considering this one this is 1720 mostly picked and 1718 for ketone.

And due to the aldehyde also have CH bond on the Sp connected. So, they have a if you see here 2720 and 2820 2700 to 2800. If you look at carbonyl of ketone this hydrogen is missing. So, this band you do not get here. So, that is one of the significant difference you could differentiate between a aldehyde and ketone based on IR spectrum.

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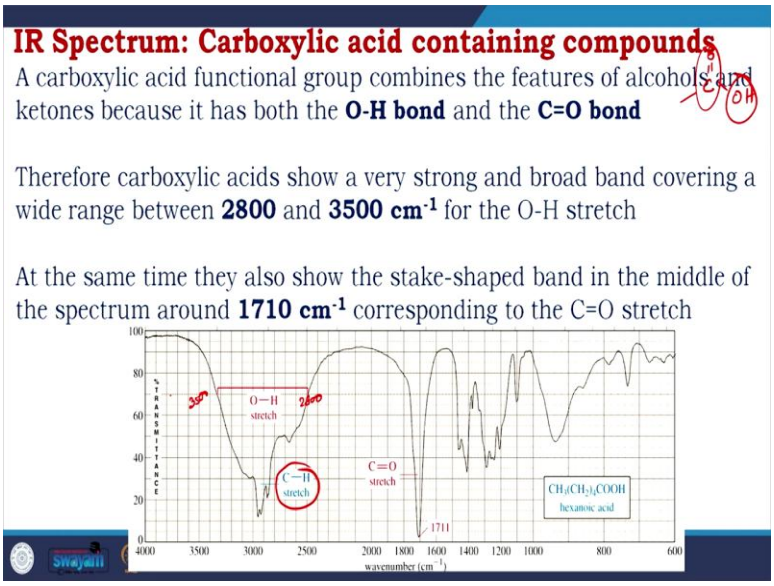

Coming to carboxylic acid: So, this is carboxylic acid containing compounds. So, if you see carboxylic acid you get a carbonyl you get a hydroxide. So, you get this OH and you also get the CO these are the main ones but you also get a CH stretch here. A carboxylic acid functional group combines the features of alcohols and ketones because it has both the hydroxyl bond and the carbonyl bond. Therefore carboxylic acids show a very strong and broad band covering a wide range between 2800 to 3500 centimeter inverse here.

If you see around 2800 to 3500 at the same time they also show the stake shaped band in the middle of the spectrum around 1710 centimeter inverse corresponding to the CO stage. So, this is 1711 which is 1720 for aldehyde and 1718 for ketone.

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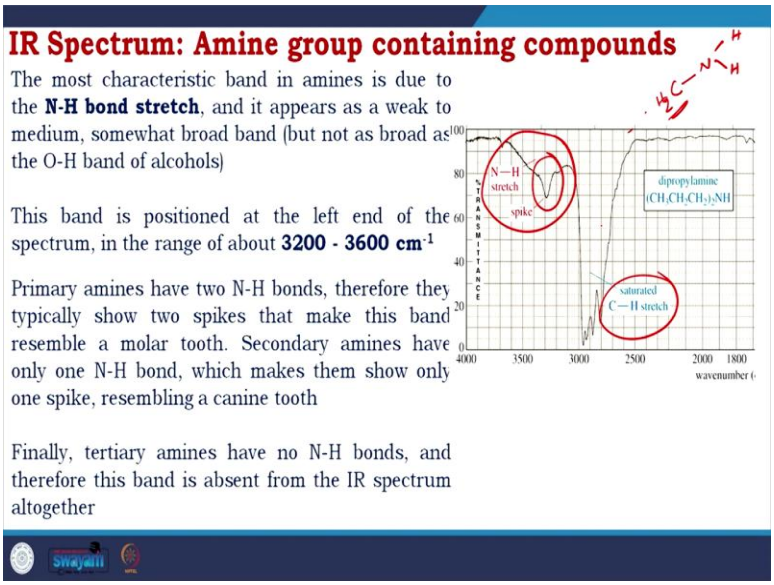

Coming to amine group containing compounds: So, if you see amine, now get NH stretch. So, you get them the NH stretch in this region you also get saturated CH stretch because it comes with other carbons. So, you get CH stretch here. The most characteristic band in amines is due to the NH bond stretch and it appears as a weak to medium somewhat broad band but not as broad as the hydroxyl group. So, this is weak the polarity or the dipole moment is not.

So, high this band is positioned at the left end of the spectrum in the range of about 3200 to 3600 centimeter inverse. Primary amines have 2NH bonds therefore they typically show 2 spikes that make this band resemble a molar tooth. So, this is what they got the spike. Secondary amines have only one NH bond. So, you could differentiate between primary amine and secondary amine because you get the 2 and 1 and tertiary NO.

So, primary amines have 2 NH bonds therefore they typically show 2 spikes that make this bond resemble a molar tooth secondary amines have only one NH bond which makes them, so, only one spike resembling a canine tooth. Finally tertiary amines have no NH bond and therefore this band is absent from the IR spectrum all together.

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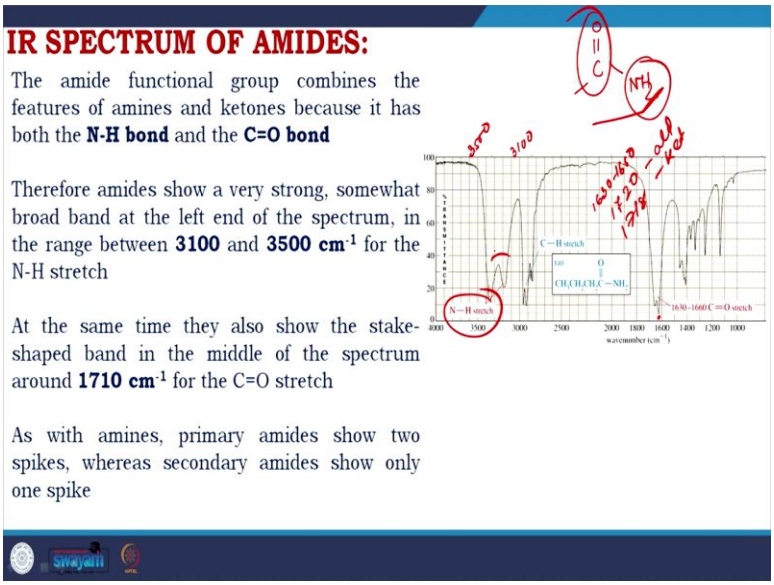

Coming to amides: So, amide, the amide functional group combines the feature of amines and ketones because they have the keto group and amine group. So, the amide functional group combines the feature of amines and ketones because it has both the NH bond and the CO bond. Therefore amides show a very strong somewhat broad band at the left end of the spectrum in the range between; 3100 to 3500 for the NH stretch coming from here.

At the same time they also show the stake shaped band in the middle of the spectrum around 1710 centimeter inverse for the CO stage. If you remember, so, they are showing it around here in this compound they are showing around 1630 to 1660. So, we have seen in other compounds aldehyde 1720 ketone 1718. So, this is also a characteristic one as with amines primary amide. So, 2 spikes whereas secondary amides show only one spike.

So, we have look at this NH stretch and we get 2 pipes because this is a primary amine for secondary amine we will get only one. Again one thing to add here that amide band have other importance as you know the peptides in protein which is our goal have this amide group. We will see later there are amide 1 and amide 2 characteristic bands which help experimentalist detect the secondary structure of the protein using IR spectroscopy.

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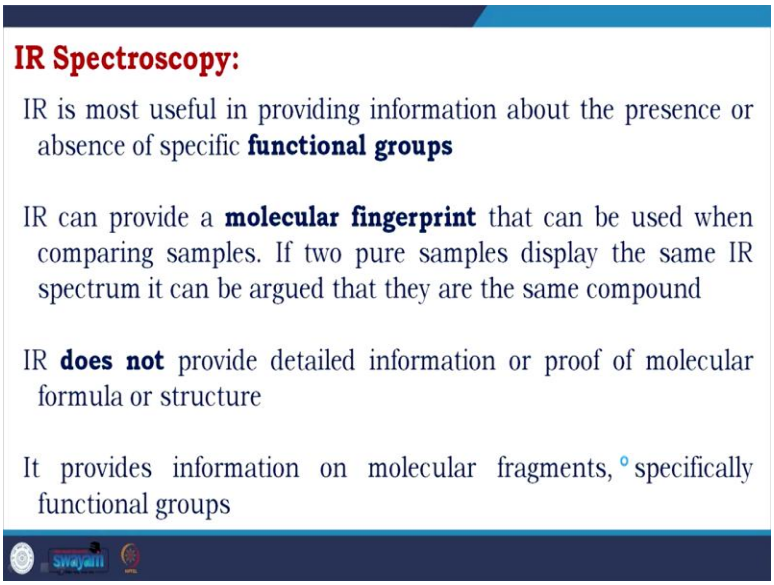

So, as discussed here and we have looked at different compounds infrared spectroscopy is useful in providing information about the presence or absence of a specific functional groups. IR can provide a molecular fingerprint that can be used when comparing samples if to pure sample display the same is spectrum it can be argued that they are the same compound. IR does not provide detailed information or proof of molecular formula or structure like NMR or other techniques.

It provide information on molecular fragments especially functional group as we have seen the hydroxyl group the amide group the carbonyl group all have particular stretches particular peaks and that would help us to identify those functional groups presence in a experimental sample.

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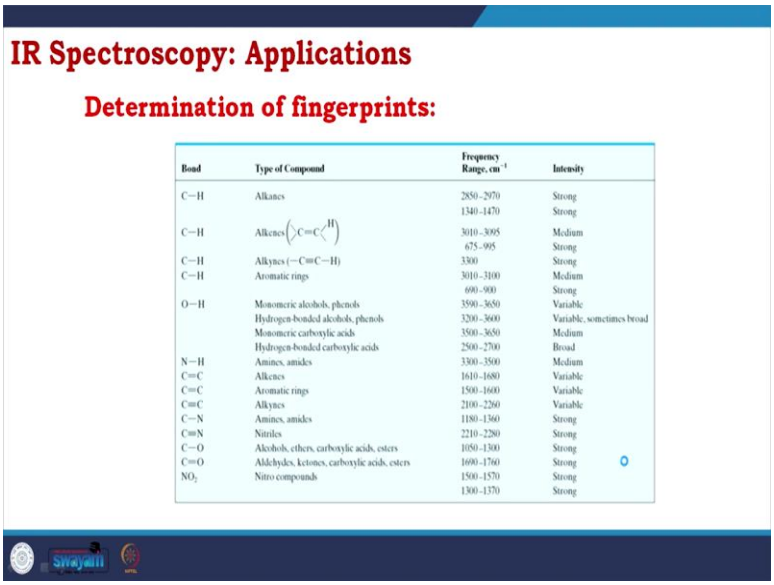

Application: Infrared spectrum is applied for compositional analysis of organic, inorganic and polymers because as we have discussed continuously they are excellent in identifying the functional groups. First application in biological and biomedical fields for example detection of water in biological membrane for biological sample also where we do not know about the solvent and all we will look at that later.

Analysis of aircraft exhausts, measurement of toxic gas in fuels, combustion gas analysis all could be done with very good precision using infrared spectrum. So, application determination of fingerprints: So, if you see for alkenes they have stretches 2850 to 2970 strong 1340 to 1470 strong for alkenes the double bond again they have spectrum. So, for CH stretch for OH stretch for NH stretch C double bond C, C double triple bond C, C single bond N, C triple bond N, C single bond O, C double bond O, N O 2 for all of them.

They have fingerprints they have a range of frequency and if in the sample this functional group are present we take the sample do the experiment get the peaks and match it with them.

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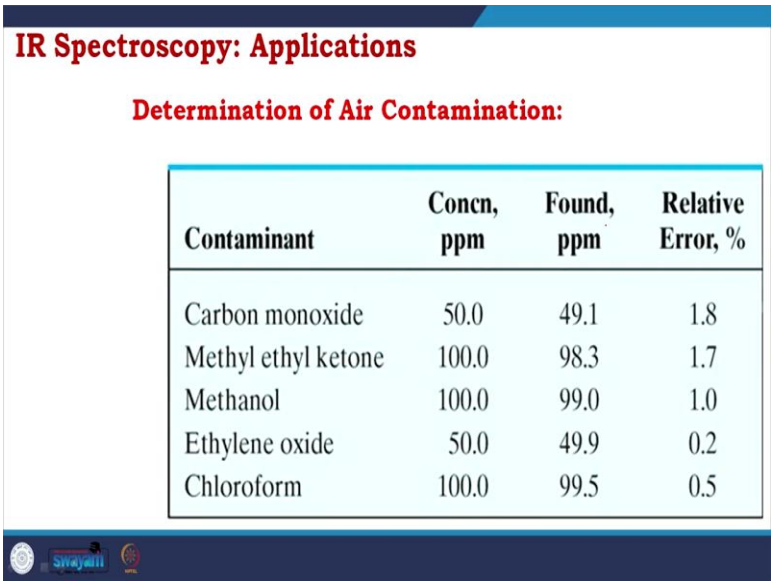

Detection of unknown sample this is a very good technique for detecting unknown samples like if you see we have a spectra of a unknown sample and we have the library of known standard compounds. Now you match them if it match you know that what type of sample it is like if you see here there is a unknown sample we plot this sample against absorbance in the y axis and wave number in the x axis and you get the spectra.

The spectra exactly match with benzene. So, we know that this unknown sample is benzene. It is very easy very convenient in detection of unknown sample determination of air contamination. So, if you see the data we have carbon monoxide, methyl ethyl ketone, methanol, ethylene oxide, chloroform and you get the concentration in ppm. You found the presence and from there you could have calculated the how much air contamination happened.

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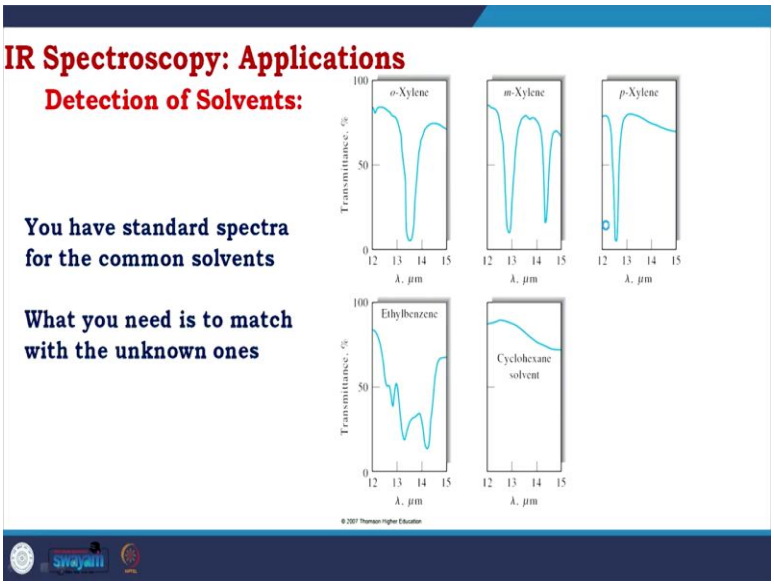

Solvent again these are characteristic spectro, ortho xylene, metazylene, paragyline ethyl benzene cyclohexane. So, all of them are solvent you have standard library of this solvent. So, when a new sample comes and you do not know the solvent you match the spectra and you determine the solvent. So, you have standard spectra for common solvents you develop a library and then what you need is to match with the unknown one the you get the spectra of the unknown one and match in the library,

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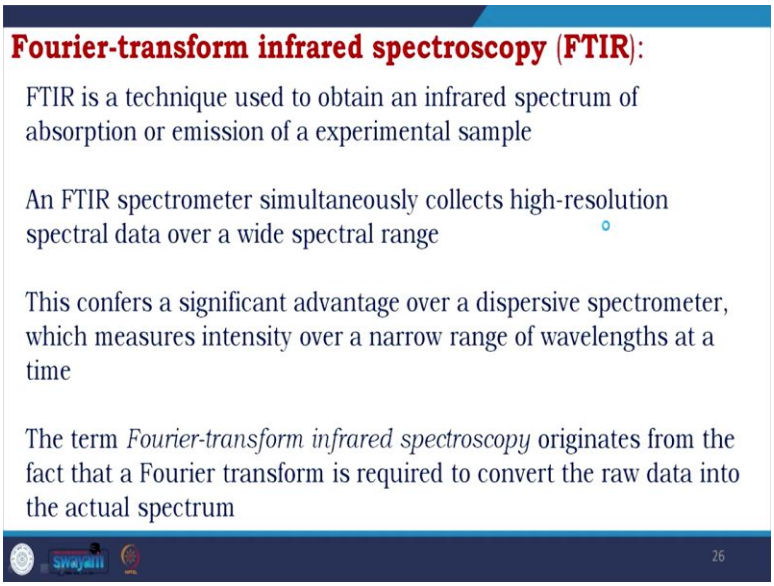

Fourier transform infrared spectroscopy FTIR: FTIR is a technique used to obtain an infrared spectrum of absorption or emission of an experimental sample. An FTIR spectrometer simultaneously collects high resolution spectral data over a wide spectral range. This confers a

significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at times.

So, more, broader or wide spectral range you get more generalized detection or generalized application the instrument could do. As we know the term Fourier transform infrared spectroscopy originate from the fact that Fourier transform is required to convert the raw data into the actual spectrum. So, we are now going to the automation. So, the analog data is now converted to the digital data and that makes the data processing quicker the analysis more first convenient accurate and that is why a Fourier transform infrared spectroscopy is used.

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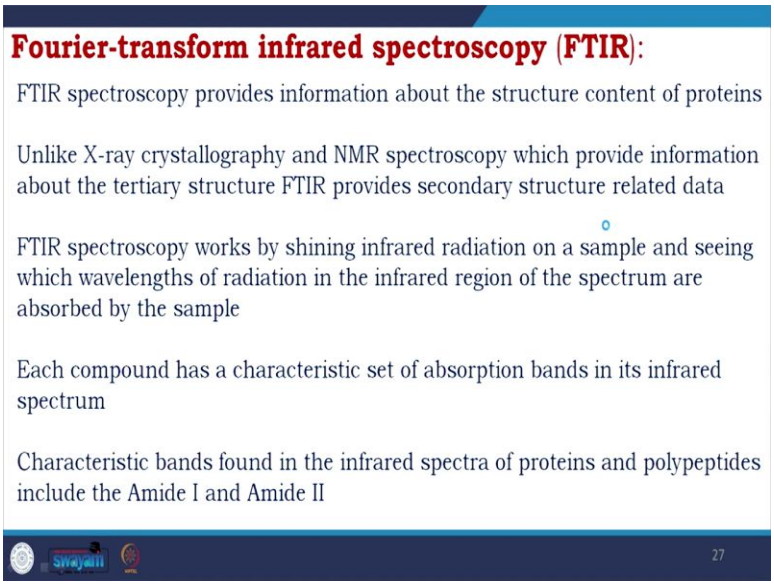

FTIR spectroscopy provides information about the structure content of the protein. But when I say structure content of the protein unlike extra crystallography which we; discussed NMR spectroscopy which we discussed which provides information about the tertiary structure of the protein. FTIR provides only secondary structure if you remember our NMR module I have explained how NMR makes its journey in determining the secondary structure of the protein and then go to the determination of the tertiary structure of the protein.

Whereas if you consider the technique of x-ray you will clearly see that x-ray directly go to the determination of the 3D structure of the protein. So, NMR determined today structure Fourier transform infrared spectroscopy also determine 2D structure. So, how it works FTIR

spectroscopy works by shining infrared radiation on a sample and seeing which wavelength of radiation in the infrared region of the spectrum are absorbed by the sample.

As we have discussed as a general in the IR each compound has a characteristic set of absorption bands in its infrared spectrum. Characteristic bands found in the infrared spectra of protein and polypeptide which include amide one and amide 2 band.

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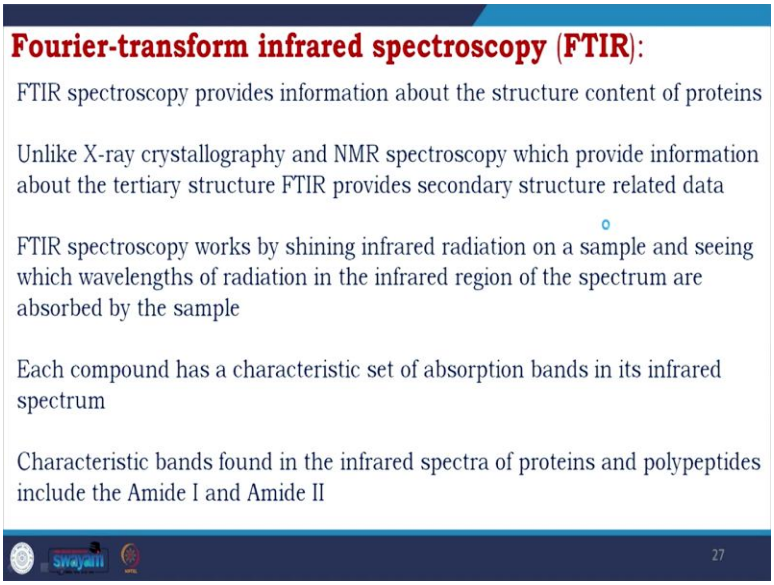

And now the amide 1 and amide 2 band this arise from the amide bond that link the amino acid. If you see here amide 1 vibration and amide 2 vibrations these are the cause of development of amide 1 and amide 2 IR band. The absorption associated with the amide 1 band leads to stretching vibration of the carbonyl bond of the amide. Absorption associated with the amide 2 band leads primarily to bending vibration of the NH bond.

So, because both the carbonyl and the NH bonds are involved in the hydrogen bonding that takes place between the different elements of secondary structure. The location of both the amide 1 and mi 2 bands are sensitive to the secondary structure content of the protein. What this is talking about. So, as I told this is the amide 1 and this is the amide 2 depending on the how the hydrogen bonds are developed depending on that there will be change in the stretching and bending that would tell you the position or the sensitivity of the characteristic part of the protein and how this could be related to the secondary structure.

Studies with proteins of known structure have been used. So, already there are study of the known protein structure and they help to develop the fingerprints here. So, actually some are fingerprints which we say fingerprint they are characteristic. Some are not characteristic they are altering but you take a lot of known samples you collect you record the data you have your own library and now matching with the library you can comment on the unknown protein that is what happening here.

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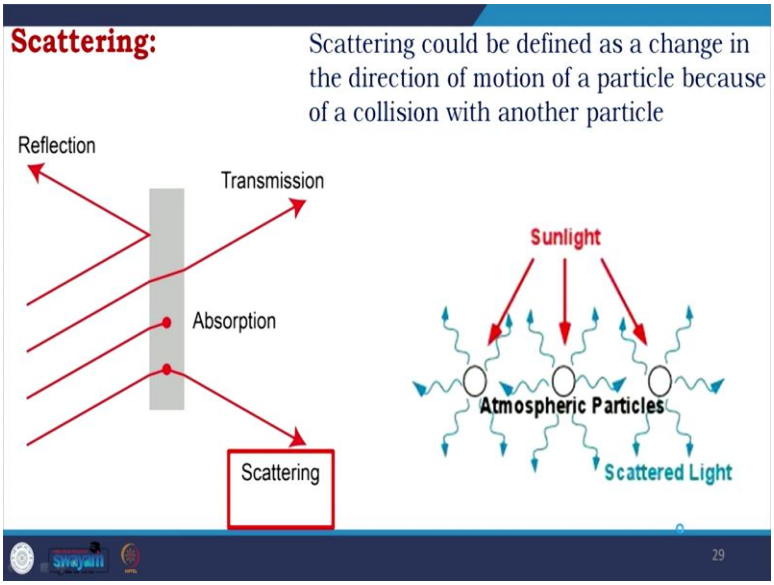

So, I will now shift to scattering you know I talked about the interaction of matter with light it lead to many phenomenon's like reflection, transmission, absorption, scattering. And as we have discussed earlier the basic foundation of spectroscopy is the interaction between the light and the matter. So, now we will talk about scattering. Scattering could be defined as a change in the direction of motion of a particle because of the collision with another particle. If you see the sunlight comes and hit the atmospheric particle and then it scatter.

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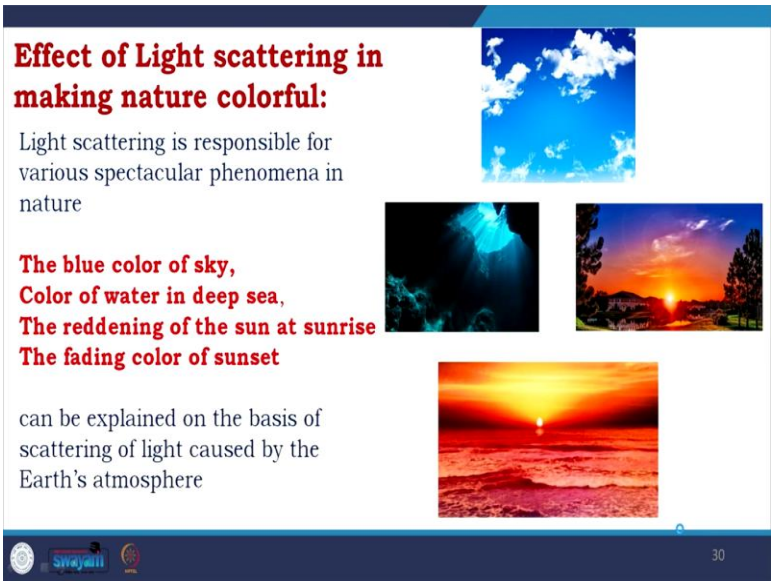

What is the effect of light scattering in making on nature? So, light scattering is responsible for various spectacular phenomena in nature. The blue colour of the; sky if you see the bright blue sky. The colour of water in the deep sea you see the beautiful colour. The reddening of the sun at the time of sunrise you see and the fading colour of sunset all of them actually can be explained on the basis of scattering of light caused by the earth atmosphere.

So, here nature photographer next time when you are publishing nice photograph please do not forget to acknowledge the effect of scattering on making your photo more beautiful.

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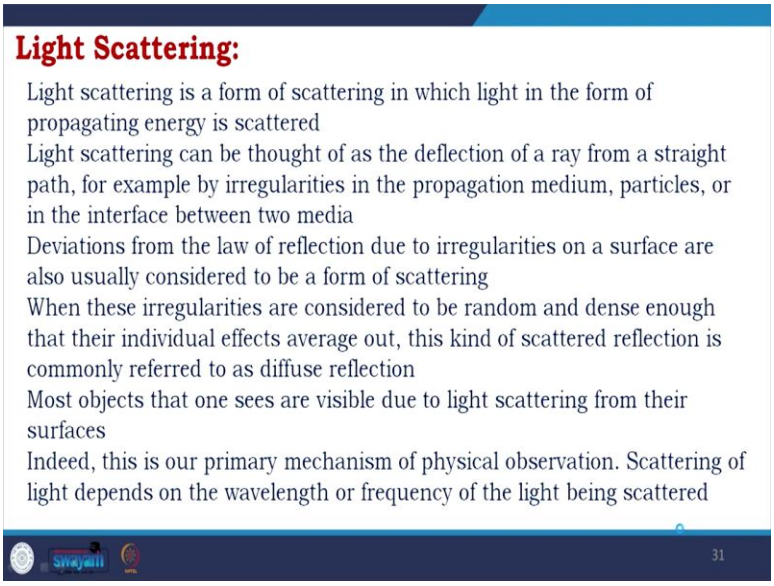

So, light scattering is a form of scattering in which light in the form of propagating energies scattered. Light scattering can be thought of as the deflection of a ray from a straight path for example by irregularities in the propagation medium particles or in the interface between to media the phase change the deviation from the law of reflection due to irregularities on a surface are also usually considered to be the form of scattering.

When this irregularities are considered to be random and dense enough that their individual effects average out this kind of scattered reflection is commonly referred to as diffuse reflection not scattering here. Most objects that one sees are visible due to light scattering from their surfaces indeed this is our primary mechanism of physical observation scattering of light depends on the wavelength or frequency of the light being scattered.

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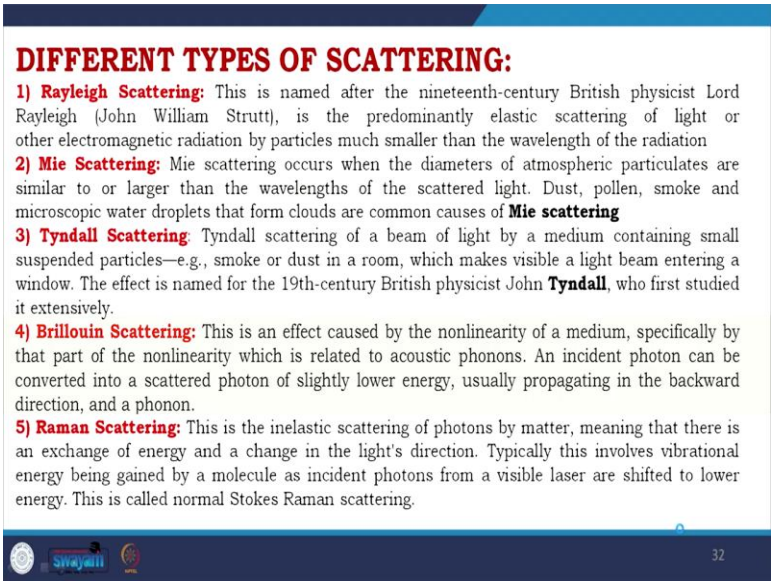

There are different type of scattering like Rayleigh scattering which is named after the 19th century British physicist Lord Rayleigh it is the predominantly elastic scattering of light or other electromagnetic radiation other than light by particle much smaller than the wavelength of radiation. My scattering: My scattering occurs when the diameter of atmospheric particulates are similar to or larger than the wavelength of the scatter light.

Dust, pollen, smoke and microscopic water droplets that form clouds are common cause of my scattering. Tyndall scattering: Tyndall scattering of a beam of light by a medium containing

small suspended particles. For example smoke, dust in a room which makes visible a light beam entering a window the effect is named for the 19th century British physicist John Tindall who first studied it extensively.

Brillouins scattering this is an effect caused by the non linearity of the medium specifically by that part of the non-linearity which is related to acoustic phonons. An incident photons can be converted into a scattered photon of slightly lower energy usually propagating in the backward direction and a phonon. Raman scattering this is the inelastic scattering of photons by matter meaning that there is an exchange of energy and a change in the light direction.

Typically this involves vibrational energy being gained by a molecular incident photon from a visible lesser accepted to lower energy this is called normal stokes Raman scattering. And because it is due to the vibrational change Raman spectroscopy is very comparable to higher spectroscopy and that is the reason we are studying here. So, though there are different type of scattering our today's discussion is on Raman scattering.

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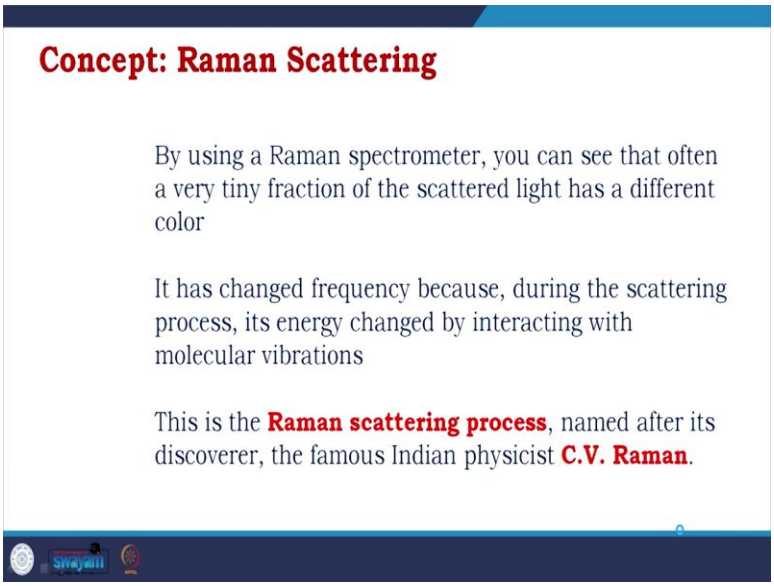

So, by using a Raman spectrometer a spectrometer which was specifically designed or you could say fine tuned by C. V. Raman the Nobel laureate. So, that you can see a very tiny fraction of the scattered light has a different colour. It has changed the frequency because during the scattering

process its energy change by interacting with the molecular vibration. This is the Raman scattering process named after its discoverer the famous Indian physicist C. V. Raman.

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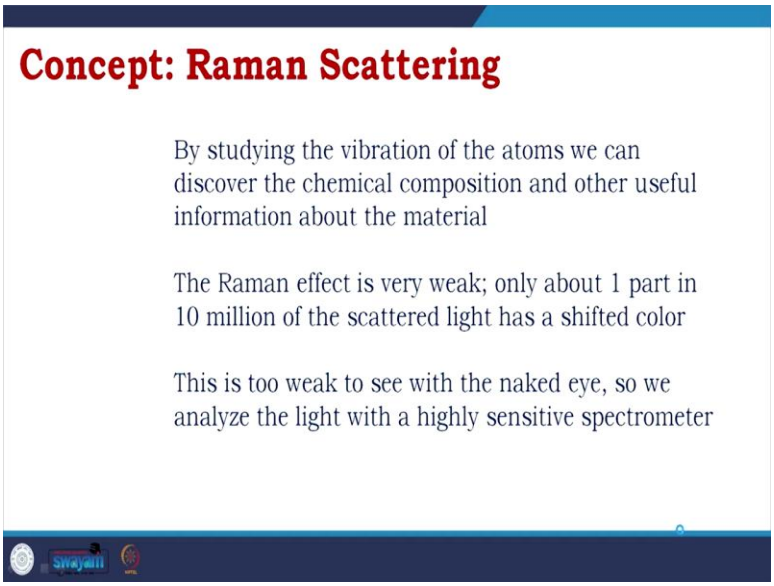

By studying the vibration of the atoms we can discover the chemical composition and other useful information about the material. The Raman Effect is very weak only about one part in 10 million of the scattered light has a shifted colour. This is too weak to see with the naked eye. So, we analyze the light with a highly sensitive spectrometer.

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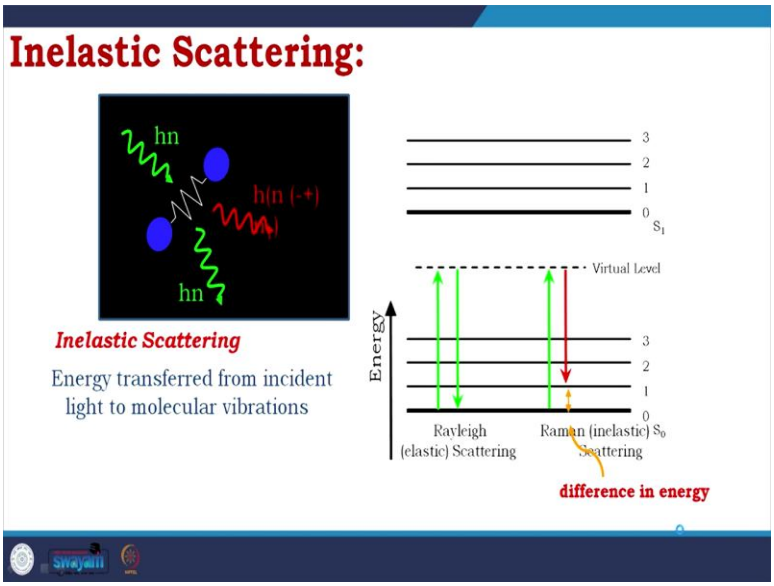

So, we are talking about inelastic scattering. So, the ray comes you see and then it goes through and a general major scattering the elastic scattering is called Rayleigh scattering which we talk

about. But then there is another one with a little difference in energy this is called inelastic or Raman scattering. This is the energy transfer from incident light to the molecular vibration.

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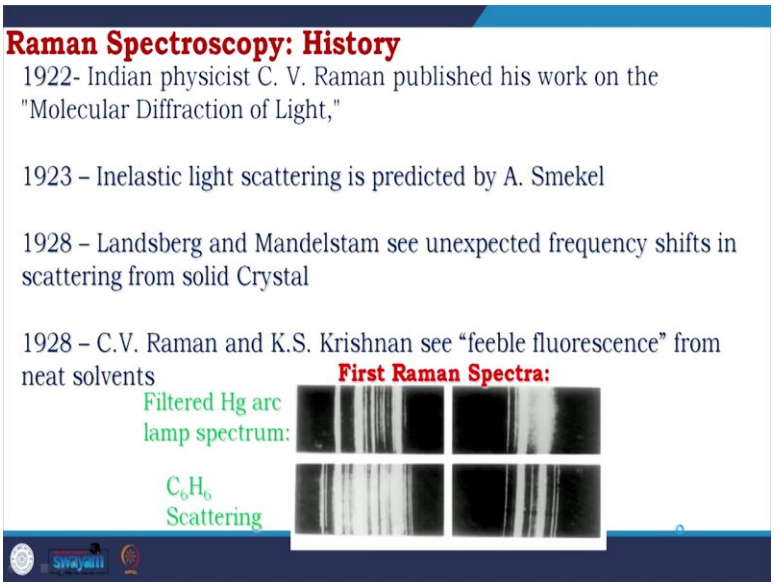

So, Raman spectroscopy is used to determine the molecular motions especially the vibrational mode of motion. Little bit coming to the history in 1922 Indian physicist C.V. Raman published his work on the molecular diffraction of light in 1923 inelastic light scattering is predicted by AS Smekal. 1928 Landsberg And Mandelstrom see unexpected frequency shifts in scattering from solid crystal.

In 1928 C.V. Raman and K.S Krishnan see feeble fluorescence from neat solvents. This is the first Raman spectra where they use filtered mercury arc lamp spectrum and you see the characteristic inelastic ones.

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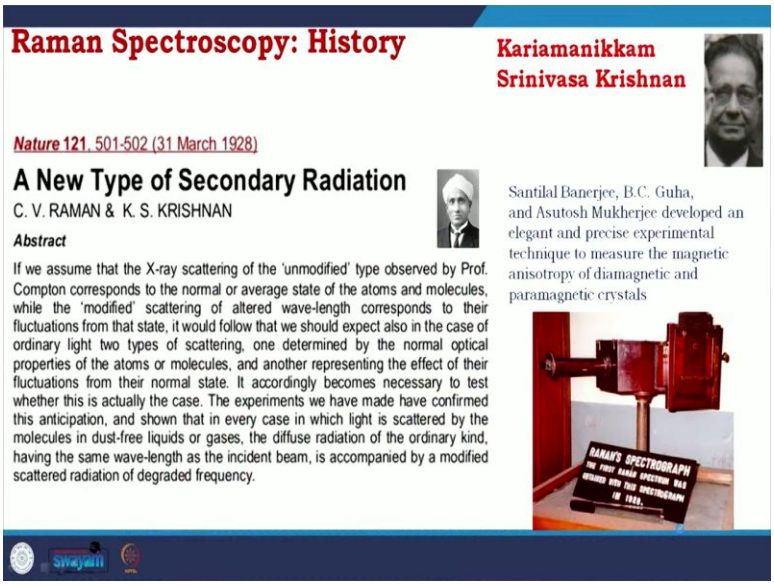

So, they published C. V. Ramen and K. S. Krishnan published this work in nature with the title a new type of secondary radiation. You all know about C. V. Raman, I will little bit talk about K. S. Krishnan. Kariya Manikam Srinivasa Krishnan he is a great physicist and we majorly known him for his contribution to this Raman spectroscopy but even being a tragic hero who did not get a Nobel prize being directly contributed to the invention. He further he was a post doctoral worker he further go to Dhaka university he joined Santilal Banerjee, B.C. Guha and Ashutosh Mukherjee and develop an elegant and precise experimental technique to measure the magnetic anisotropy of diamagnetic and paramagnetic crystals.

So, this is also a very significant contribution and K. S. Krishnan was a very humble and noble person as identified by many greats at that time including that time Prime Minister Jawaharlal Nehru. He is the first awardee of the Santi Sarub Bhatnagar award. So, this is little bit about K.S. Krishnan and this is the Raman spectrometer which we are talking about in Calcutta in the institute ISCS it is kept there as a memorial which is the first in endogenous setup of Raman's lab who have identified the spectra using this spectrograph.

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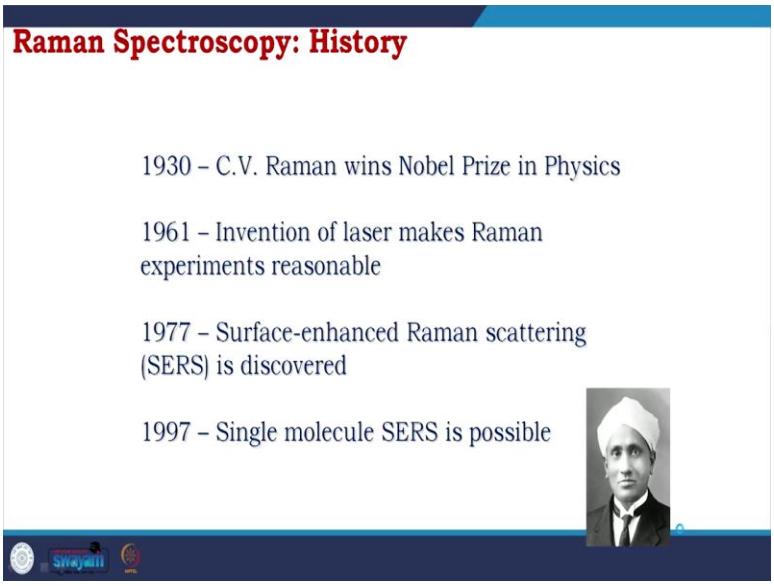

Continuing with little bit of history in 1930 C. V. Raman wins Nobel prize in physics for this contribution in 1961 invention of laser makes Raman experiments reasonable which we are going to talk about. In 1977 surfaced enhanced Raman scattering or source is discovered in 1997 with the increased sensitivity to the instrument it goes to such a level where single molecule source is also possible to study.

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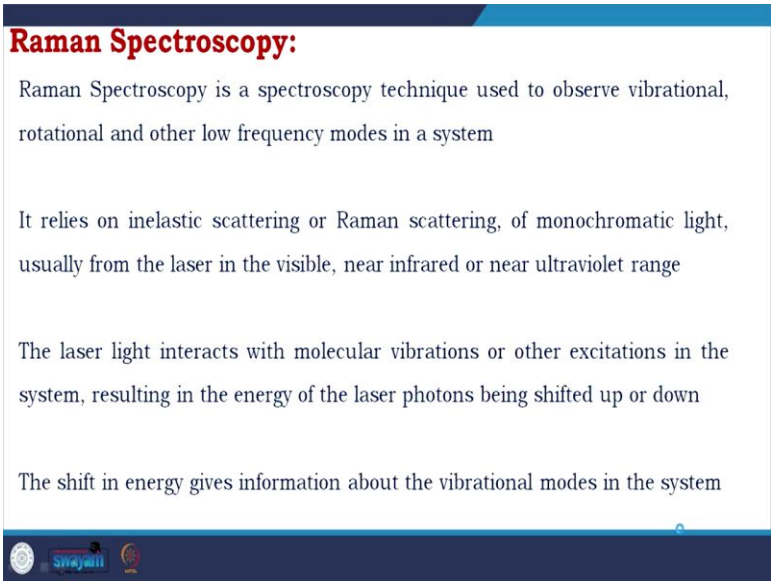

So, Raman spectroscopy is a spectroscopy technique used to observe vibrational rotational and other low frequency modes in a system. It relies on inelastic scattering or Raman scattering of monochromatic light usually from the laser in the visible near infrared or near UV range. The

laser light interacts with molecular vibrations or other excitations in the system resulting in the energy of the laser photons being shifted up or down.

The shifts in energies; gives information about the vibrational modes in the system and are very characteristic.

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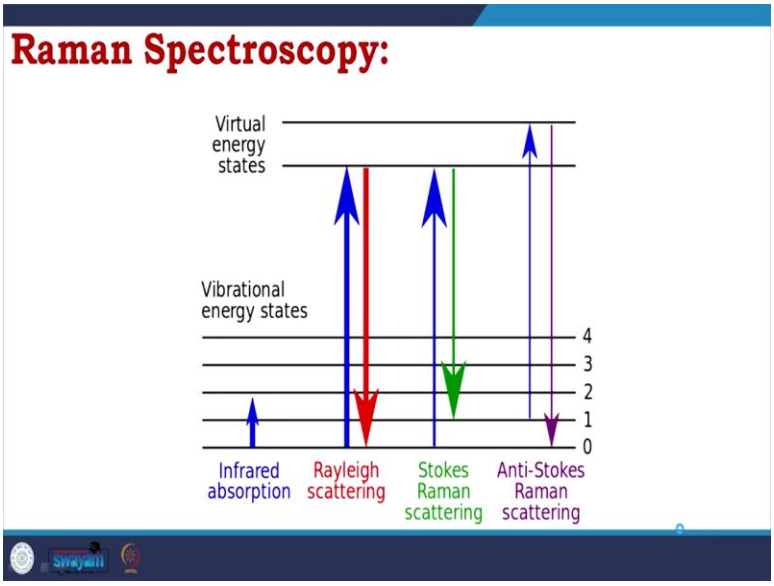

So, if you see we are talking about the in the infrared level there is elastic or Rayleigh scattering and for Raman its strokes and anti-strokes.

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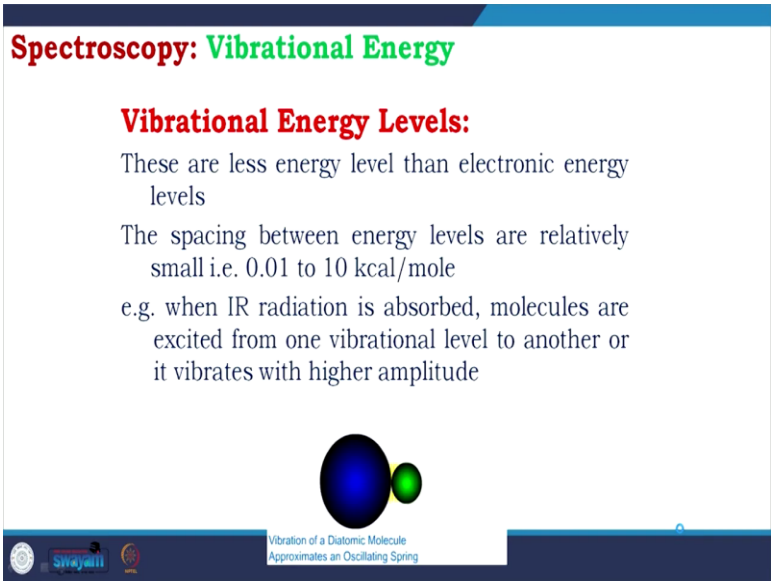

So, we have discussed but vibrational energy levels these are less energy level than electronic energy level in between we talked about when you are introducing spectroscopy. The spacing between energy levels are relatively small 0.01 to 10 kilo calorie per mole when IR radiation is absorbed molecules are excited from one vibrational level to another or it vibrates with higher amplitude. So, this is vibration of a diatomic molecule it approximate an oscillating spring as I talked about.

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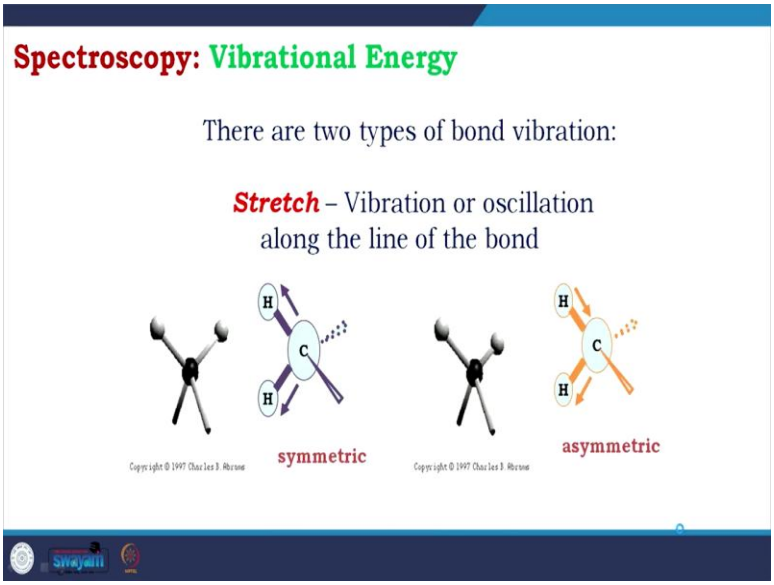

So, there are 2 type of bond vibrations again I talked about them stretching, vibration or oscillation along the line of the bond they could be symmetric as you see here both are having a symmetry and asymmetric. If you see there is getting small and getting bigger. So, that is asymmetric.

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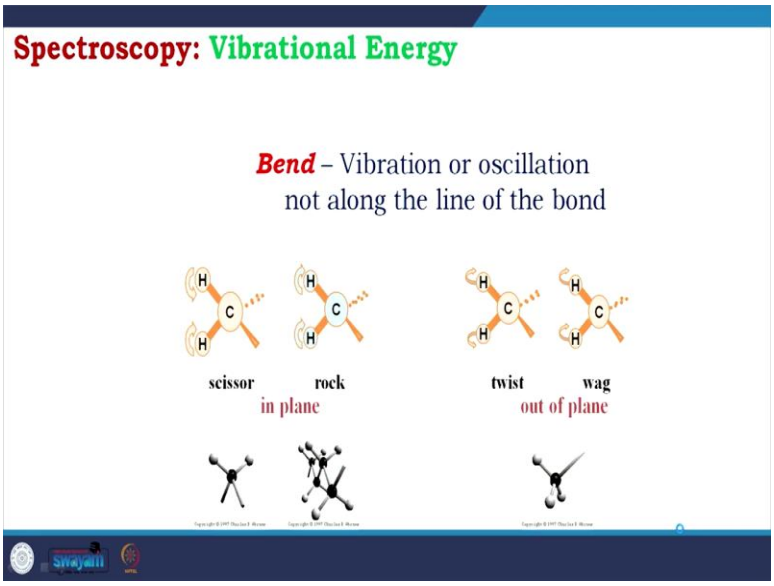

And then bending, vibration or oscillation not along the line of the bond which are in plane it calls scissor and rock out of the plane it called twist and work. These animations help you to understand the difference between scissor rock and twist.

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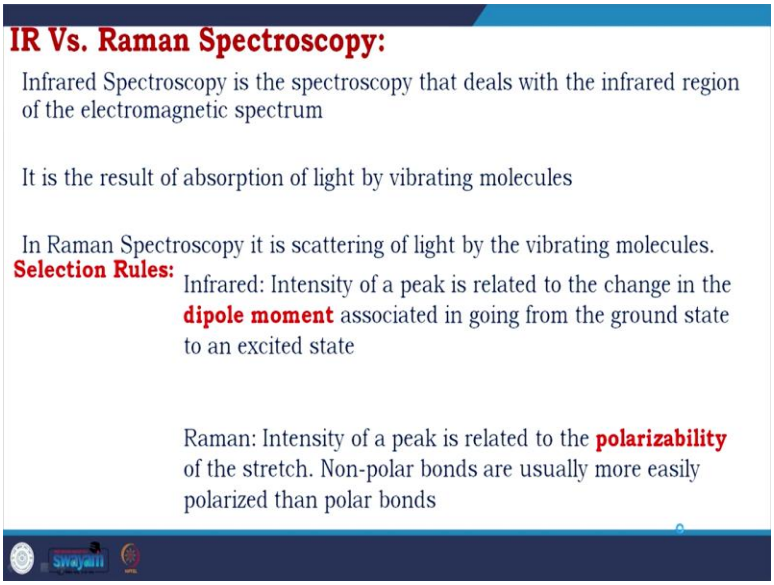

Infrared spectroscopy is the spectroscopy that deals with the infrared region of the electromagnetic spectrum. So, as I told both IR and Raman they both operate on the vibrational level. So, comparison is extremely important. So, IR spectroscopy is the spectroscopy that deals with the infrared region of the electromagnetic spectrum. It is a result of absorption of light by vibrating molecules. In Raman spectroscopy it is scattering of light by the vibrating molecules.

So, we compare them because both comes as a outcome of the effect of the vibration of the molecules. There are selection rules infrared the intensity of a peak is related to the change in the dipole moment associated in going from the ground state to an excited state. In Raman, intensity of a peak is related to the polarizability of the stretch non polar bonds are usually more easily polarized than polar bond.

So, that is where the key where we find these 2 technique as competent to each other. So, here in case of higher more dipole moment stronger the band in Raman the; nonpolar bond have more tendency to polarize and the polarizability is measured. So, that is where you get a very nice you know co-relation between the 2 technique. And that would be done by mutual exclusion principle.

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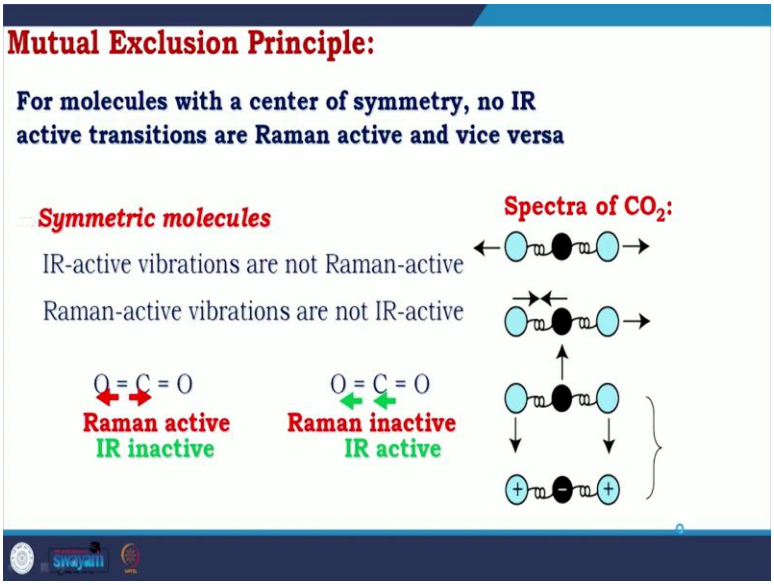

So, for molecules with a center of symmetry no IR active transitions are Raman active and vice versa what that means. So, symmetric molecule IR active vibrations are not Raman active and Raman active vibrations are not IR active. So, we are taking the molecule carbon dioxide. So, if you see when it goes in 2 directions it is Raman active when they are going in one direction it is Raman inactive. When it is Raman active it is IR inactive when it is Raman inactive it is IR active.

So, if we look at spectra of carbon dioxide as I told when it goes in the 2 direction you get a Raman 1335 centimeter inverse and when it get in the head to head direction you get IR 2349 centimeter inverse when it get like in up down you get IR 667 centimeter inverse.

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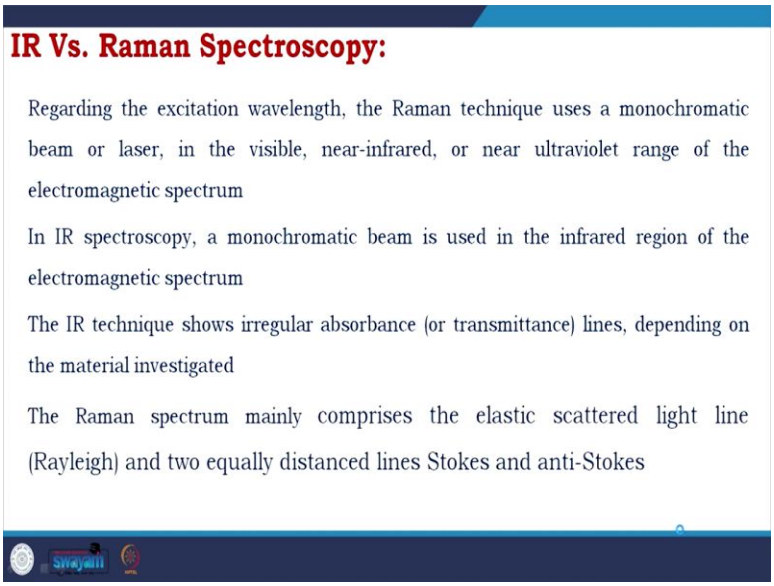

IR versus Raman spectroscopy regarding the excitation wavelength the Raman technique uses a monochromatic beam or laser in the visible near infrared or near ultraviolet range of the electromagnetic spectrum. In IR spectroscopy a monochromatic beam is used in the infrared region of the electromagnetic spectrum. In the IR technique shows irregular absorbance lines depending on the material investigated.

The Raman spectrum mainly comprises of elastic scattered light line Rayleigh and to equally distance line strokes and anti-strokes.

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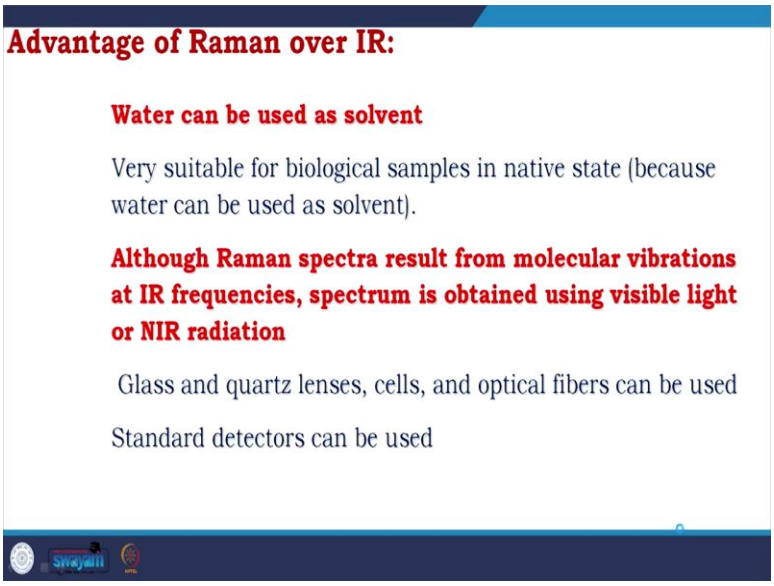

Advantage of Raman over IR, water can be used as solvent. So, very suitable for biological samples in native state because here; you could use water as a solvent. Although Raman spectra result from molecular vibration at higher frequencies spectrum is obtained using visible light or in IR radiation. Glass and quartz lenses cells and optical fibers can be used standard detectors can be used that the advantages of Raman.

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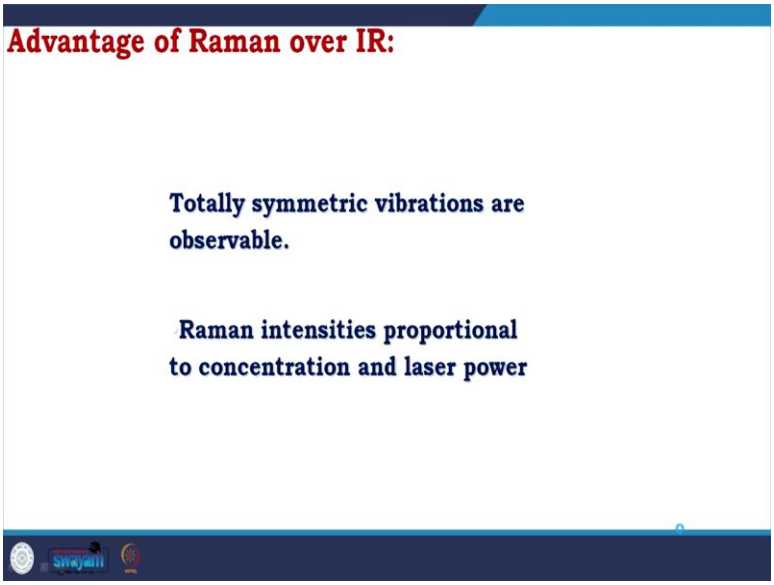

Totally symmetric vibrations are observable; Raman intensity is proportional to concentration and laser power so, easy to calculate.

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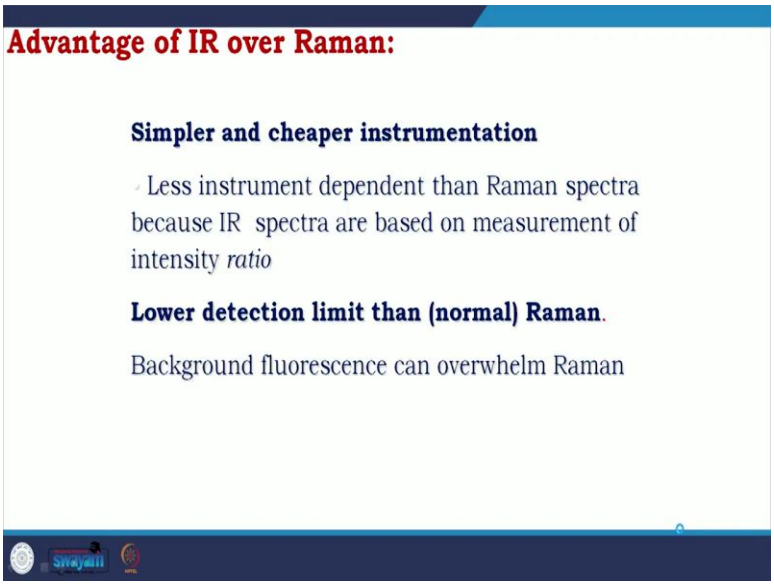

Now advantage of IR over Raman: Simpler and cheaper instrumentation, less instrument dependent than Raman spectra because IR spectra based on the measurement of intensity ratio, lower detection limit than normal Raman. We are not talking about resonance Raman that is coming. So, we are talking about normal Raman the detection limit of IR is lower. The background fluorescence can over help normal Raman due to its weak signal. More suitable for vibration of bonds with; very low polarizability because with low polarizability you cannot measure them by Raman.

So, we have talked about the basic of IR spectroscopy and how it is applied to getting spectra and how to identify the functional groups which have number of applications and they also have some fingerprints which are very convenient to detect presence of a lot of small molecules. Then we have shifted to Raman compare between IR and Raman. And we also talked about how IR work over the identification of the secondary structure of protein.

In the next class we will discuss about Raman spectroscopy and its application in studying protein and a very interesting and a novel instrument setup which is called Raman microscopy and Raman crystallography. We will talk about them and we will also talk about how this Raman crystallography would be utilized towards development of a detection of a live experiment. This is a very smart and novel setup where you could study a real enzymatic reaction how it is going

on how it is changing the molecule or the compounds chemistry. We will see that in the next class, thank you very much.