Multidimensional NMR Spectroscopy for Structural Studies of Biomolecules

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Lecture – 02

Energy levels in NMR spectroscopy: Quantum mechanical model and Vector model

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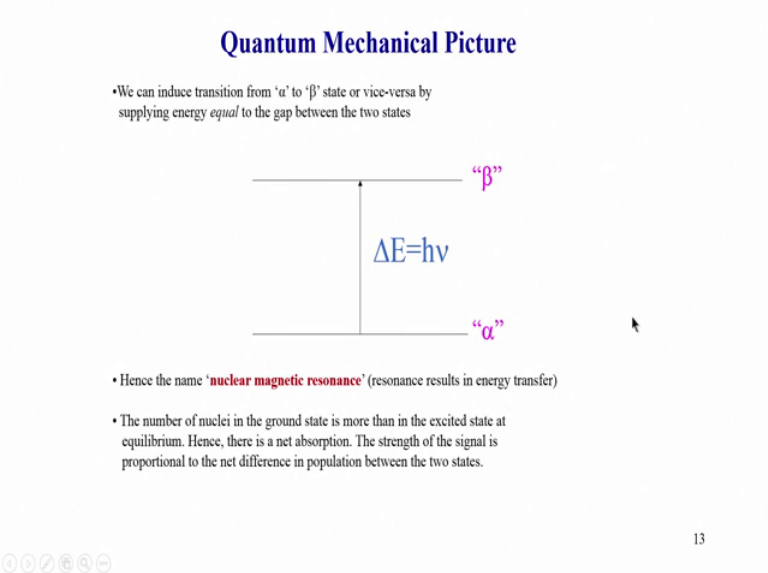

Welcome back. In the last lecture, towards the end, we saw that there are two energy levels which are created in spin half nuclei by applying a magnetic field. And I mentioned that the number of molecules which are in the ground state are higher than the number of molecules which are in the upper state, excited state. Now, the question is how do we know that what decides whether molecules are more here or equal or higher in the upper state.

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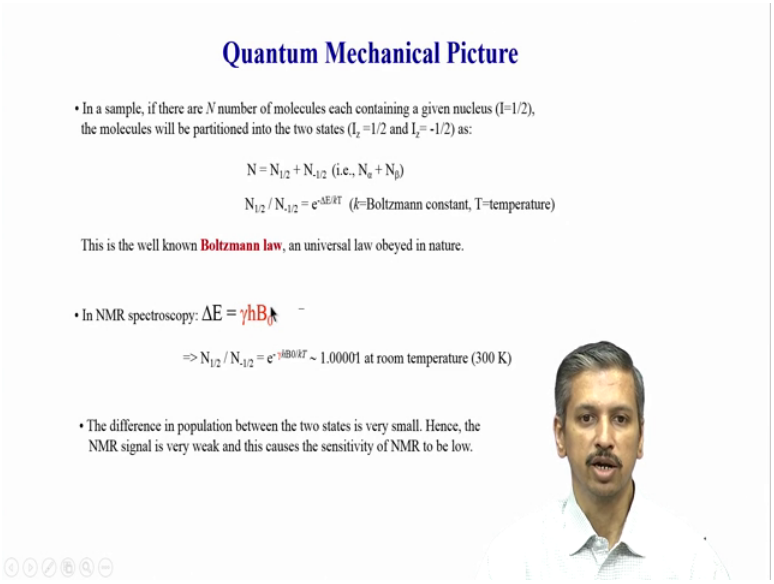

So, there is a famous law which is called as a Boltzmann law which is what we now look at; and that helps us to decide or tell what is the ratio or what is the number of how many molecules are in the ground state and how many are in the upper state. So, let us go through that a equation. So, let us say we have half the number of molecules which are in the ground state, let us call them as N half and the molecules which are in the excited state we will call them as minus half, because I will be remember beta state is higher energy and the plus half alpha state is lower energy.

So, total number of nuclei or sample molecules is N alpha plus N beta. It is the Boltzmann law is given like this, the Boltzmann formula. It says that the number of molecules in the upper state if you divide it by the lower stats the ratio, if you take the ratio of number of molecules, it is given by e raise to the power minus delta E over k T, where delta E is the energy difference between the two levels. This is the energy gap which I showed in the last slide. So, what is this energy gap?

Now, energy gap is created because of the magnetic field that means, if I have a very high energy gap that this number will go down if you can see this, if I put higher value of delta E, e raise to minus delta E by k T will come down which means the number of molecules in the upper state will go down because this ratio will decrease. So, this ratio therefore is dependent on this value of delta E other parameter which it depends on is this temperature because k is a constant.

So, if I decrease the temperature, if I decrease the temperature, you can see here in this if you put the formula that lower temperature, this the number of molecules the ratio will actually again go down; which means the number of molecules in the ground state will become much more than the number of molecules in the upper excited state. So, lower the temperature the ratio is lower, meaningless number of molecules are in the upper. If in the limit that you take T equal to 0 that is a 0 degree Kelvin remember this is in Kelvin's not in Celsius units.

So, if you take temperature as 0, you will see that the entire number of molecules are in that. So, this actually is essentially the Boltzmann formula which helps us that the energy that depends on basically the difference in the energy gap. And that is this energy gap which I just now showed in the previous slide is given by this formula gamma h into B 0. So, it depends on gamma. So, the bigger the gamma higher the gamma, the energy gap will be higher. And similarly higher the magnetic field you apply, it will be higher. So, the magnetic field and gamma control the energy gap between the upper state and the lower state.

So, this is something which we used to play which are we NMR spectroscopy is use this to play around to increase the sensitivity or decrease and so on which we will see as we go on. But remember look at this ratio here, it is not that it is really a big difference, the two upper state in ground state are almost equal. You see just 1.00001 10 to the power minus 5 more than in the ground state is little bit more than the upper state ok. So, this is the ratio of upper state to ground state, it is opposite of what I said, but that ratio is little only little more than 1; which basically means that the populations are almost equal between the upper and lower, but not exactly equal, there is still more in the ground state. And that is a reason why we say that NMR is very low in sensitivity.

And where is the sensitivity coming from the why is the sensitivity dependent on this, it depends on the difference in the molecule number of molecules between the ground state and the upper stage. So, the difference if it is very small, you can see here the difference is they are almost equal. So, the difference is very close to 0. And if the difference is 0, exactly 0, of course, the sensitivity is 0. But if the difference is very small, the sensitivity is also very small, so that is one of the reason why there has been a lot of push in that NMR technology to improve this increase this delta E.

Remember this is something not in our hands. This is a constant for proton, carbon and nitrogen and any nucleus. For what can be changed is this B 0. So, if I increase the B 0 going to higher and higher magnetic field, I can increase delta E, and if I can increase delta E I can improve this ratio, I can make it much little bit better than 1, I mean little bit better than what is it right now at room temperature. So, therefore, there was been lot of technological push to go for higher magnetic field and very especially for biomolecules like proteins and nucleic acid its very critical that we go to very high magnetic field.

The reason being the sensitivity is a very key factor. In biomolecules the sensitivity is always low because the sample is the big molecule then proteins are big molecules, the concentrations are very low. We normally do not get very high concentration samples because they either aggregate or they the availability is low. So, under such conditions we have to go for as high sensitivity as possible. And that the only way to do that is one of the ways I have to do that is go to higher magnetic fields.

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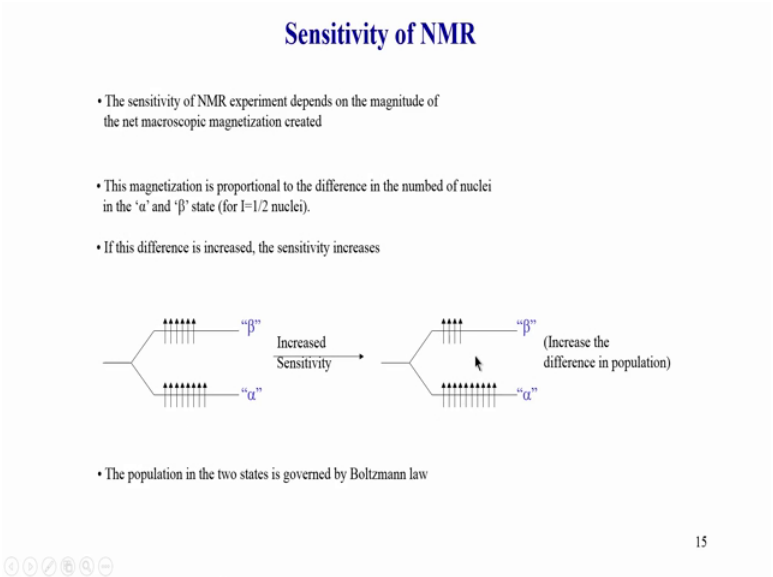

So, as we go along we will see how this magnetic field affects the sensitivity. So, this is what is now in this particular slide, you can see here that the difference between the molecules. Now, here this an exaggerated difference. It is not actually what we normally see, but this is just as a schematic drawing. So, you can see there is a more there are

more number of molecules in this alpha state, there is a lower energy state compared to the higher energy.

Now, how do I increase the sensitivity? I can increase sensitivity if I reduce the number of molecules here shown here, and I increase the number of molecules in the ground state. So, what am I doing therefore, I am the actually increasing the difference in the population. So, what is important for us is this difference. So, if I keep decreasing this side and I keep increasing on this side, my sensitivity will keep increasing. But I cannot do that simply by changing some parameter I have to actually change either the magnetic field which is depending on this gap; that means, a I have to make this gap more and more or I have to lower the temperature.

So, typically what happens in NMR is temperature is something we do not go below for biomolecules especially we do not go below 0 degree Celsius because that is a freezing point for water which is the most common solvent in biomolecules. So, therefore, going below 0 degree Celsius which is 273 Kelvin is not an option. So, mainly the temperature is ruled out. It has a option to increase the sensitivity the only option now is to increase the magnetic field, so that is why as I said magnetic field higher the magnetic field better the sensitivities. So, that is essentially what is coming from the Boltzmann law there that that the population difference depends on the magnetic field and the temperature.

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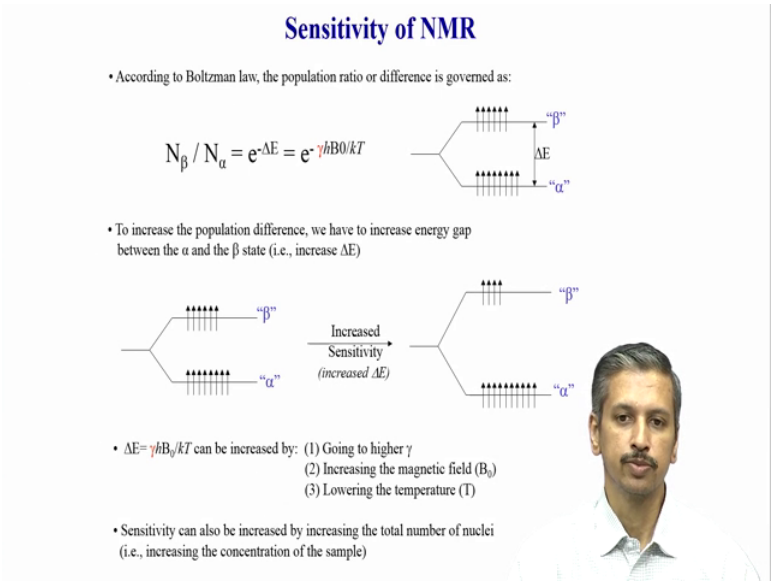

So, this is shown here again that if we actually now let us look at the temperature parameter how temperature affects. So, you can see that here if I increase the delta E and keep the temperature constant. So, I am keeping this constant, but I am increasing this magnetic field. If I do that, I increase this gap. When I increase this gap, according to this formula, my number of molecules in the N beta that is upper state will go down, because delta E is e value should be delta E by k t. So, this is what we shown here delta E by k T.

So, if I increase the temperature as or increase the magnetic field, this term comes down I mean this whole term become less which means N beta is less. So, the ratio becomes less. So, if there are more molecules here than the upper molecule. So, this is one of the very popular ways to increase sensitivity NMR and especially for biomolecules which we will need to which we will see as we go along.

So, these are the three different ways you can actually increase the sensitivity, we have seen if you can go to higher gamma which is essentially given to a nucleus which has higher gamma value the most highest gamma is for hydrogen proton and this is why we hydrogen is the most popular nucleus. And carbon is not very high it has 4 times less gamma than proton. So, carbon sensitivity is much less than proton.

So, again for biomolecules, there are different ways to improve that, one option is to use we will come to that that is called hetero nuclear NMR methods where you transfer the energy from hydrogen to carbon that is one way or you can actually make the carbon enriched the molecule completely enrich be c-13 because c-13 has two problems one is it has lower gamma compared to proton. And its abundance c-13 abundance is also very low, it is only 1 percent. So, at least we can increase the abundance of c-13 by enriching the molecule and that comes under the purview of isotope labelling and that will help us to improve.

A second way to our option to increase the sensitivity, we already saw that is magnetic field which increases the gap, and all of them actually increase the gap. So, therefore they increase the mole population difference. And lowering the temperature, this as I said is more or less not so possible for biomolecules, the reason being we take biomolecules normally in water most of the time and water samples freeze at 0 degree Celsius. So, obviously you cannot study a frozen sample a molecule, so that is why we have to work normally at room temperature.

We can actually increase the concentration of the sample that is another way to improve the sensitivity, but the concentration cannot be again increase too much for biomolecules, because they start aggregating, they start denaturing or degrading. So, therefore, there is a limit on the concentration, what we can do for biomolecules. So, as we will see few slides down further other parameters for sensitivity, which is important.

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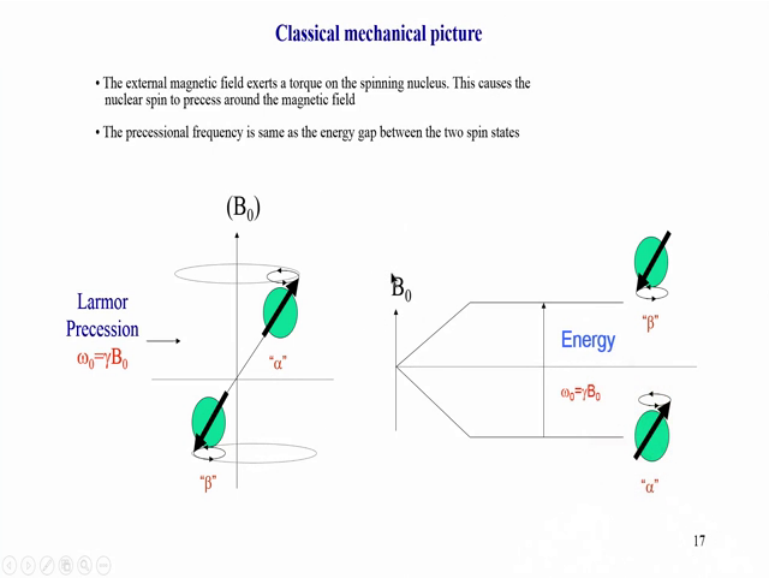

Now, let us look at now little bit deeper into this concepts of NMR. So, as I said we have already seen this that picture on the right is familiar now, we applied a magnetic field. And that magnetic field resulted in a energy gap, and we apply energy radiation. And that radiation is given by this frequency, and that takes a molecule from a ground state to the excited state.

And this is the what is known as a quantum mechanical picture, why do we call it as quantum mechanical picture, because remember when we talk about only in quantum mechanics we talk about discrete energy levels. So, this discrete energy levels meaning they are not continuous, there is a finite gap and that discreteness has come because of applying the quantum mechanical rules.

Now, NMR is a beautiful technique which not only can be understood in this way, it can also be understood in a purely a simple hand waving vector picture or what is called as classical way classical picture ok. So, this is something which is very unique to NMR. Normally, when we talk about spectroscopy, it comes under quantum mechanical way of

describing it, but NMR can also be understood in a very little in an hand waving I would say or a vector model.

So, let us see how we can do that how we can understand NMR in this manner that is in a classical manner. So, in classical picture what we say is that the NMR, we just say something which I will not derive in this part, and this is was done in the previous course, and also it is available in textbooks. So, the result is that that a nucleus which is spinning. Now, it has two types of weaker motion, one is the spin which already we used to called it as half h cross or half or integer or i value, where i was basically half for hydrogen, so that i we can think of it has a spinning nucleus.

Now, the nucleus also starts moving round this magnetic field, and that is called as a Larmor precession, that happens because of some physical principles. And that the nucleus now, we can say if it is this molecule there is a nucleus hydrogen is in alpha state, it will precess like this; and if it is beta state, it will precess in the anticlockwise or in the opposite manner ok, so that is essentially that two states which are depicted here.

Now, this precession means it is like molecule going around this axis needs, we have to give a frequency value to this, because this has a particular speed at which it is moving. And that frequency is given by omega equal to gamma into B 0. This is omega is basically the frequency or angular frequency is equal to gamma which is a gyro magnetic, and B 0 is a magnetic field. So, what it says is that this movement that is this precession is faster much faster, if I increase the B 0; and this precession is very slow, if I reduce the B 0.

So, this precession is basically dependent on the magnetic field, and what is interesting is this frequency value which is essentially gamma B 0 of the precession is also coming here, you can see this is the same as energy needed for it to be excited. So, there is now you can see some connection coming out between the classical way of looking at this NMR phenomenon, and a quantum mechanical way. We actually will now exploit this connection to explain or to understand the phenomenon of resonance so as we go on. So, what is important to very an important notice that this precessional frequency that is just the frequency of the angular frequency of the movement is the same as the frequency, which is needed for energy for the molecule to jump from ground state to energy state.

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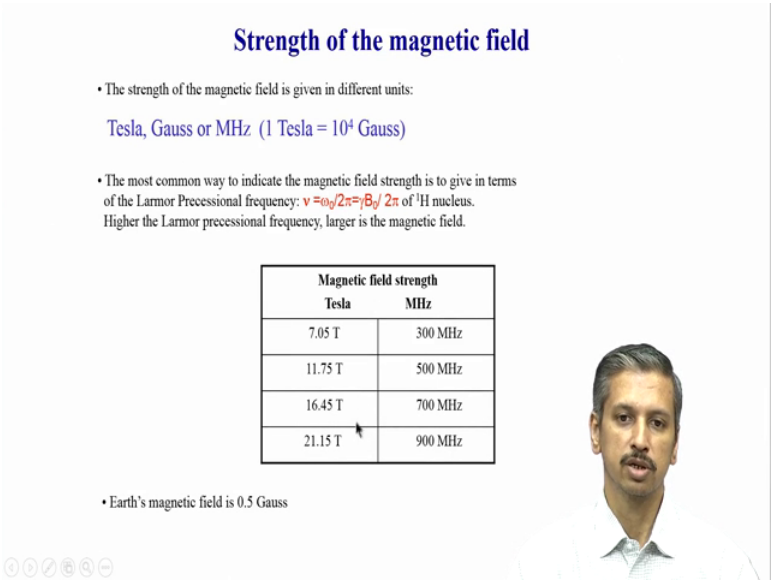

So, you can see this frequency omega is now if given in some units which is megahertz, because there the magnetic field is also used in units of tesla. And if you plug in the values something which you can see here has been done, so if you let us say your magnetic field is seven tesla, and if you put this value and gamma value here, gamma value something which is available in all the textbooks. So, you should try this yourself put this take the value of gamma for hydrogen for example multiply that with the magnetic field, which is in tesla you will get this value that is three hundred megahertz.

So, this is the frequency which you always we mention of the spectrometer a 300 megahertz spectrometer, a 500 megahertz spectrometer and so on. So, that value is coming from the magnetic field. So, you can see now for 11.75 tesla, it is 500 megahertz. And if we go to very high magnetic field 21 tesla, it is 900 megahertz.

So, when again biomolecules we deal with this kind of magnetic fields or this kind of magnetic fields, and for small molecules we will look at smaller magnetic field. So, now how do we when I say it is a huge magnetic field how do I know it is a huge, it is actually comparison with earth magnetic field. So, if you look at earth magnetic field, this is the one which is normally present which means all around us it is 0.5 gauss.

And you see here 1 gauss 10 to the power 4 gauss is 1 tesla 10,000 gauss is 1 tesla not only that we have 21 tesla as a magnetic field. So, 21 into thousand 10,000, you see it is

a huge high very high magnetic field compared to what is normally regular NMR int the in the earth magnetic field.

So, therefore we are we have to be very careful about this magnets, and they are very specially built, they are not simple magnets, they are superconducting magnets. And there are lot of important things behind the construction of magnet, which we may not be able to see in this course, because this course mainly focuses on application and the hardware NMR aspects of this course of the magnets have been discussed in the previous course. So, I would recommend to look at those slides or those videos for understanding the magnets.

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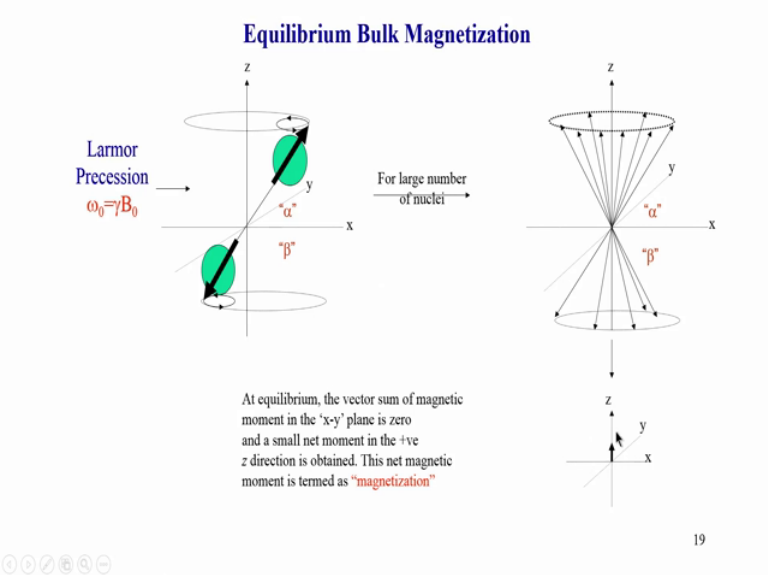

Now, let us come back to this classical picture here. So, now as I said NMR can be understood both in classical way as well as in quantum mechanical way for a large for easy understanding this picture is much better. So, now let us say that this is alpha state which is pointing in the up direction, and this is a beta state in the negative direction. And they are precessing with the Larmor precession, and that frequency as I said is omega naught we use a word 0 here is because the main magnetic field, which is applied along z axis is normally denoted as B 0. So, B 0 and therefore omega is omega 0 ok.

So, if B 0 becomes B 1, then omega will become omega 1. So, this 0 which is shown here is follows this 0 here. Now, we consider only one or two nuclei hydrogen, but in a in a sample it is not like that. In a sample you have a large number of nuclei. Because, if

you for example I will consider water, you will have 10 to the power 23 Avogadro number of molecules of water, and each water molecule has 2 protons.

So, you see the protons will be now really a huge number. And each proton is shown here like an arrow. So, instead of writing this kind of a picture, which is difficult to write for a large number of molecules I have returned I am showing this as a single arrow. So, one arrow represents one hydrogen atom or nucleus.

So, you can see now one thing which happens is that these hydrogen atoms are all processing around this, but they are not together, they are actually spread out, meaning they are not going they are all moving in the same direction, but not moving together they are spread out, this is a random difference, but this there is no specific particular difference between the gap. So, the entire set of atoms or nuclei are spread out along the cone that is for all those hydrogen atoms or nucleus which are in the alpha state.

Remember there always some nuclei in the upper state also according to the Boltzmann law. And those are represented here by downward pointing arrows, and these arrows also are actually precessing or moving around in this direction, but again they are randomly spread out in the cone. So, the angle of this each of these arrows if you look at each of these arrows with respect to the z value z axis is same, because they are in a cone. But, they are not moving together, they are randomly face.

This is a very popular term, which we call as a random face approximation which we will come to as we go along. So, you can see here that at equilibrium equilibrium meaning I have taken the sample I have taken a sample, put it in the magnetic field. And that magnetic field creates difference and molecules are now distributed up and down, and those were in the up state are all in the randomised cone the direction, and all in the ground state are also in the randomise direction.

Now, what I can do is I can simply add the vectors in the different components. For example, I can take a component of this vector which is where there mouse is pointing along x axis, I can take a component of this vector along y axis, I can take a component of this along z axis. So, this is called components of a vector. Similarly, I can take component of this vector on the opposite side along x axis, and along y axis, and along z axis.

So, if you notice between these two, what has happened is the component along the x, and this component along this x, they are opposite. So, they will cancel each other. Similarly, if we take the component of this vector along y, we will cancel the component of this vector along minus y, so they will again cancel. But, they both of them are pointing in the z axis, the z component are in the same direction. So, they will add up ok. So, this is what happens, what happens is therefore the down the down facing spins there x and y are all cancelled, but there all z components will add up. Same thing will go in this side. Here also for each vector if I take a component along x axis, there will be somebody on the opposite side which will cancel that in the x axis.

Similarly, if I take along y axis, some component of that we will cancel along y axis. So, therefore all the molecules on the up state also have zero components along x and y, because they are all cancel each other. But, there z component will start adding up, because the z is in the all the same direction they are all pointing up. So, I have now a z component, which is pointing in the plus z axis for the alpha spins, and I have minus z a component adding up for all the beta spins.

So, now does alpha and beta starts cancelling, but they will not cancel exactly because according to the Boltzmann law, we know that alpha molecules in alpha state in the number of molecules are higher than beta state even though the difference is very small, but the difference is surely not 0.

So, therefore if I now take the z axis component of the alpha spins, and cancel with the z minus z of the beta, they will still be a finite a non-zero plus z axis component remaining, because the number of molecules in the alpha state is higher than number of molecules in the beta state at equilibrium room temperature. So, this is what we use the word in NMR magnetization. This is the net magnetic moment of net magnetization of the molecule in the sample, because now this you see this vector captures the entire spin magnetic moments, because all of them are spread like this and we have actually combined them, and then show that x and y are all cancelled, but the y remains z remains in this direction.

So, entire NMR now operates on this particular net magnetic magnetic moment or magnetization. If the population were equal, then this would have been 0. And therefore, nothing no NMR would have been possible, but the fact that populations are not equal is

what we are exploiting here, and we end up with this net magnetization. Now, this is what we actually use for carrying out all the NMR experiments.

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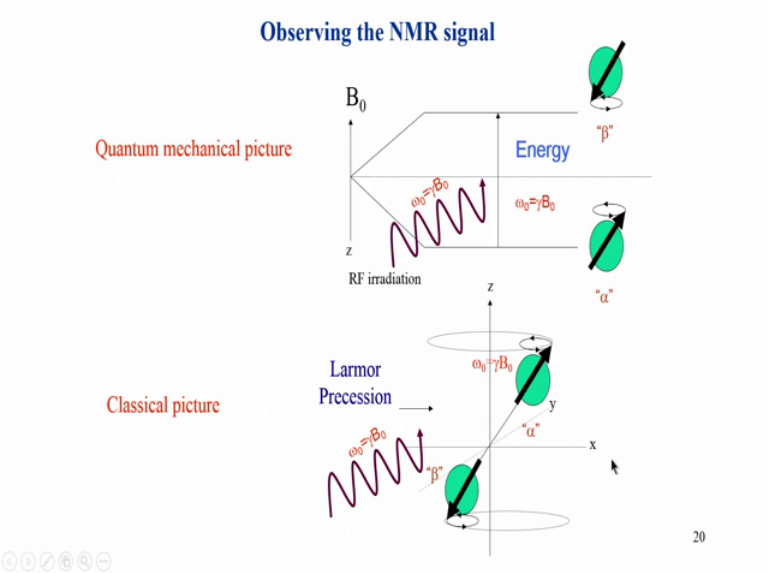

So, let us see that how we do that how do we carry out the experiment. So, this is what we shown here, this is again the two pictures classical picture in quantum. Remember NMR is a same technique, we are looking from two different perspectives two different views, a finally we have to get the same result. So, we have this picture and this picture have to co-inside of merge or be consistent with each other.

So, if you look at this picture first, what I need is an energy which will excite the molecule from ground level to the excited level, this is what I use the word resonance. For that I need to apply radiation, which matches this energy gap. And that radiation should now be equal to this energy, because that is the energy gap here. And therefore, I use omega gamma B 0, this omega gamma B 0, when they match molecules will be excited.

Now, how do I understand this whole process in the classical way, and this is shown here I do the same thing I actually have a spin which is moving in this direction, which is our Larmor precession. And I apply this radiation, which is the same with the same frequency as the precessional frequency. So, if the precessional frequency matches this energy of radiation, then this molecule will be excited, and will go from alpha state to

beta state. Similarly, beta will also be excited, it will go to alpha state. But, the entire as I said depends on how much is a population difference.

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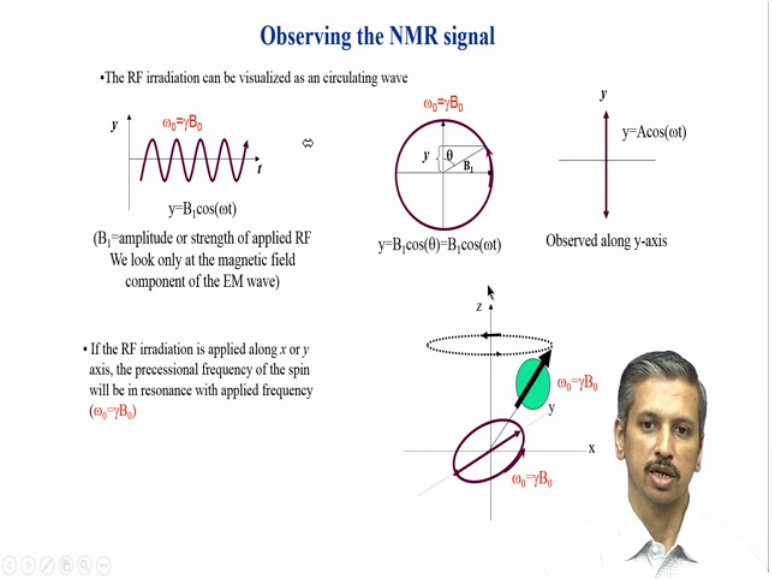

So, this is shown here in the pictorial format. This is the radiation which is been passed to the sample, and you can see this radiation can be visualised in a circle also. Remember any electromagnetic wave can also be picturise in a circular manner. So, this has what is called the amplitude, this is this length is amplitude and it is a phase. A phase essential is how much it is away from this y axis.

So, now if I apply a radiation which is and the x, y plane, so I take this circle the circular radiation, I put it in the x, y plane. So, look at this picture here, it is actually happening in the x, y direction is not in the z direction, but the molecule is moving in the z.

So, if you look at this picture here, this is also an x, y plane, because the molecule is precessing in the around the z axis. So, this dotted circle which is shown is actually in the x, y plane ok, and that is this is also x, y plain. So, these are actually parallel to each other. So, therefore if there is a resonance means the frequency of this matches the frequency of that, then we enter into the resonance condition, and the molecule now switches from alpha state to the beta state.

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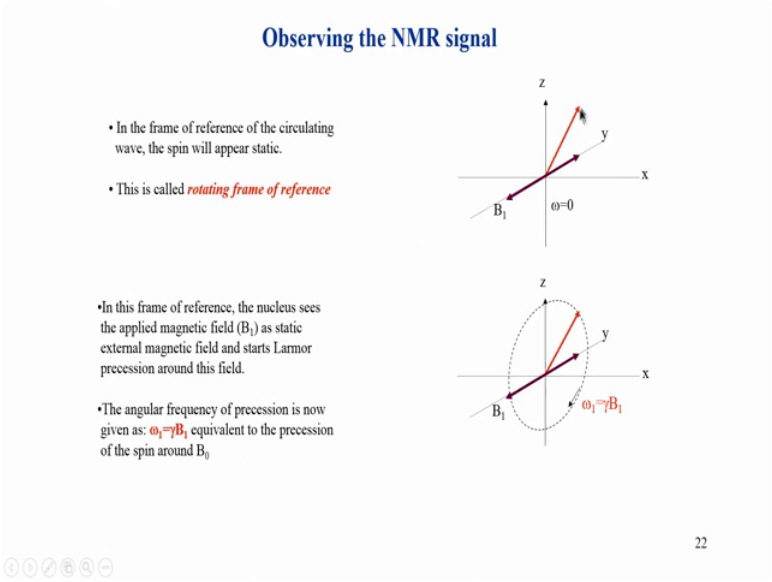

So, this is what we shown here, we can see here this arrow which is shown here. Now, in this we are using a very important concept call rotating frame of reference. I would recommend you to actually read this in the books which I have mentioned, because this is a very settle concept and if we try to show it here.

So, what is happening is if you go to this slide again, what is shown here is this this circular wave is parallel to this rotational frequency precession. And if their frequency is match, we can actually go into a frame of reference in which the frequency and this vector a static, because they are having the same frequency this is precessing with the same frequency as this precession. So, both are having as if they are looking at each other in a static, they are not moving with respect to each other, so that is called a rotating frame of reference. And it is a virtual frame, it is there is not a real rotating frame, it is a virtual frame.

We are just its like the standard example is for sample if you are moving, if there is a train going at 100 kilometres per hour, if you are standing outside the train, you see the train moving at 100. But, if you are inside the train along with the train for you or if you moving another train parallel to this train with same speed, for both of you each of you are basically static, because both are in the same speed. So, you do not see each other moving at all. So, same thing here the there are the circular radiation has the same

frequency as the precession of the spin which is shown here. So, therefore if you can say that they are static with respect to each other.

So, under that condition the spin now, because this is also a magnetic field. So, if you look back here, what I am applying here is actually a B 1, it is also a magnetic field. So, my radiation again going back a few more slides, this radiation is actually a magnetic field applied ok. So, this electromagnetic radiation now starts, the spin starts rotating around this magnetic field ok. So, earlier it was rotating around this B 0 that is this axis, but no longer it was that it because we have entered into a rotating frame, where this the spinning around this z axis is over, we have stop that we have looking onto a frame where it is gone to their static.

Now, in new frame of reference, in the rotating frame the molecule now starts as a vector now starts moving around this B 1 like a merry go round. So, now is a new rotation now, this is a new precession which starts happening. And that is what we just exploited to take the spin vector from plus half to minus half.

Remember of we want to excite the molecule, we want to go from the plus half to minus half, so that can happen by simply applying a magnetic field a rotating magnetic field, which takes the spin from plus half to minus half, but this rotating magnetic field has to be synchronised or in resonance with the Larmor precession of this. So, in the next class, we will see how this excitation can result in the NMR spectrum that is a using Fourier transform, and that is a spectrum which we always look at when analysing the data.