Principles and Applications of NMR Spectroscopy
Professor Hanudatta S. Atreya
NMR Research Centre
Indian Institute of Science Bangalore
Module 1
Lecture No 03

So welcome back, today we are going to look at how NMR data is acquired and how we actually detect the signals. Before going to that, let us recapitulate, what we learned yesterday. So, one thing is we were looking at two different pictures, one is called classical mechanics picture and other is the quantum mechanical picture. So, let us start from classical mechanics, so in this picture which is shown on the screen, so the spins are actually denoted by what is called as this arrow.

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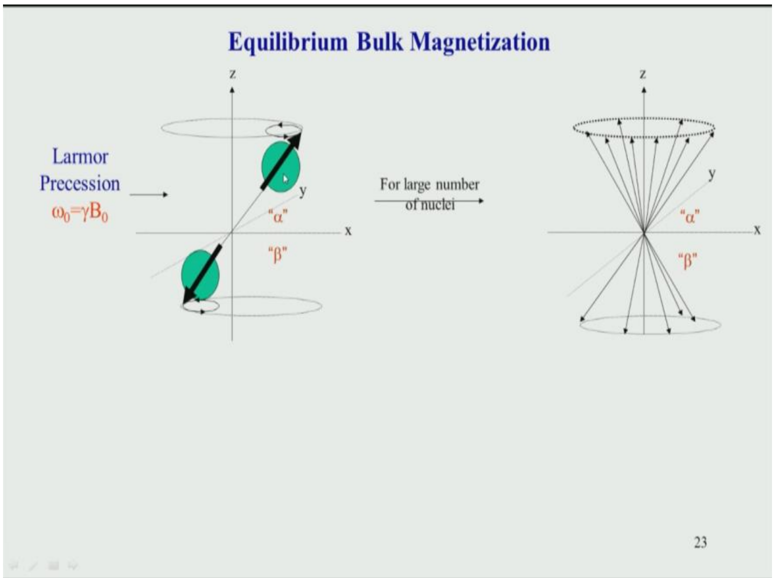

So, this arrow essentially represent one particular nuclear spin and as I said in the last class, there are two motions associated with it one is called the precession Larmor precession which is going around the magnetic field and of course other is the spin inherent spin of the each nucleus. So, now there are two possible states even you put a spin in the magnetic field, we called one as alpha state other as beta state.

So, now if you take a collection of a spin, so let us say you take a sample which has a large number of spins in each spin can now be denoted by an arrow and it will look something like this. So, here it is like a cone and all the spins are now randomly distributed around the cone and this is called random phase approximation. So, all the spins which are in the alpha state or will be in the place Z direction and on the beta state we will denote it in the - Z direction.

So, as we saw last time according to the Boltzmann law, there are more spins which are in the upper state that is in the lower ground state that is alpha state than spins which are in beta state. And therefore if you do the vector addition and subtraction, you will see that finally what you end up is with a small net magnetization pointing in the positive Z direction. So, this is what we will call as net magnetization and all the NMR experiments 1D 2 dimensional, 3 dimensional and all the experiments, now we will deal with this particular spin in in the different directions. So, now we will see how we can actually excite this spins and get a NMR signal.

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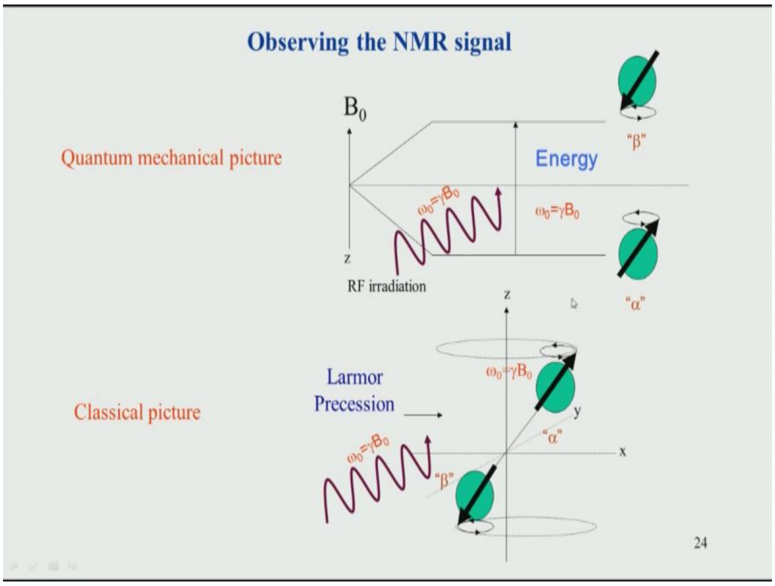

So, for exciting again we saw in the last class we use the word resonance. So, when you apply an energy which is exactly equal to the energy gap or almost similar to the energy gap, the molecules will now go from ground state to excited state that means a spin which is sitting in the ground state in the alpha state. Now will flip in the spin and then go into the beta state only when an energy is supplied, which is of the order or the same range as the gap in the energy levels.

So now how do you apply this energy this energy is applied in form of a radiation. So, this is now the next part of the NMR experiment as where you apply an energy which is called as RF pulse or RF radiation, we will see what is a pulse in the few slides some now. So you apply an RF radiation and this radiation will have a frequency which is given by omega = gamma B0 and these frequency is now = the frequency or the energy in the of the gap.

Now this frequency, so now let us look this is from the quantum mechanical picture. Now look at, let us look at the same situation in the classical point of view. So, what we are looking at is a spins are now precessing around the magnetic field with a frequency omega is gamma B0, so when we apply a energy radiation which as also has a same frequency it has this is a same frequency as this rotation of the spins we use the word resonance because now they are in the same frequency and this is called resonance. Now to understand how this particular radiation can cause that transition from ground state to upper state, we have to use a very important concept in NMR which is known as rotating frame. So, rotating frame is what we will see now, so before that let us look at how a wave can be visualized.

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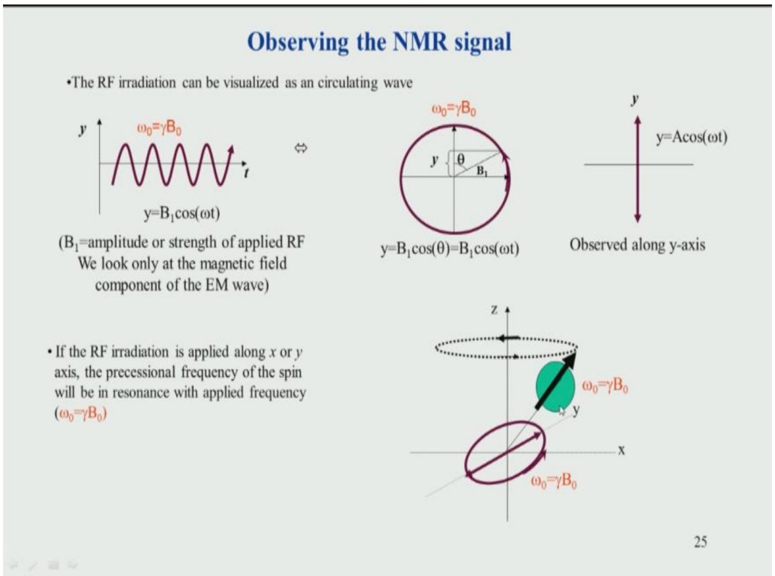

So, this is a simple electromagnetic wave radiation and we can see we are plotting here in the Y axis is the intensity or we call it as the word we use the word amplitude versus time. So this is a standard concept of a wave and it is given by this equation, where Y is the y axis amplitude = the B 1 that is the strength of the magnetic radiation multiply by cosine omega t. So, one thing we have to keep in mind here very important in the NMR prospective is that every electromagnetic wave if we discussed in the few last class, it has two components, one is called the electric field and another is called the magnetic field.

So in NMR, we do not look at the electric field, we only look at the magnetic field of that radiation of the electromagnetic radiation or magnetic field component. So magnetic field component is what is going to interact with the spins because spins are also magnets. So, magnets will interact with magnetic field and therefore we use the word B value the letter B for magnetic field. So, what we are seeing here is we have applied a radiation which is how a wave of a frequency omega and it is having amplitude B 1. Now this wave can also be visualized in a circular manner in this manner. So, this is what will be very useful, when we go through the rotating frame concept. So, you have to now imagine the wave in this particular form, so this is same as this so this is two are called interconvert I mean this is basically the same picture shown in the different format.

So, here it is showing like a wave is oscillating in a circular manner and you can see that it is the B1 is now this the amplitude this particular this vector and it is now moving around in circle, so this is called a phase. So, it accumulates the phase with respect to times, so this concept is omega into t is called a phase. So this is very similar to what is shown here, so for example, when the theta theta is 0 it is along this axis that is y is full 100% then it goes slightly goes to the x-y plane, then it comes here the y component becomes 0 because it is now exactly along x axis and the x component maximum and then it goes down, it comes to -y - x and so on.

So, this is how the wave is circulating and this is how we can visualize this RF radiation, we applied to the sample, so on this picture on this slide what is shown is the component. Suppose, let us say you are observing the component along y axis, when the component is basically somebody is, for example is is sitting here if this point where the arrow is pointing and is looking at the wave, what he will see is that initially the amplitude is maximum, then as the wave is going to the x plane the y component slowly decreases it goes to 0, then it goes to the negative y then again it comes back to 0 a goes to positive y, so basically it is something like an oscillation here. So, that it is what we say that it is applied along y axis, so let us say that we again now look at the classical picture, so this is our spin which is rotating along the z axis.

So, it is around the z axis this is a magnetic field, so now this rotation if it is synchronized with this rotation in other words if I apply an RF radiation whose rotation is synchronized in resonance with this particular spin, then we can then this is it causes resonance or it causes excitation. Now for let us look at the concept of rotating frame of reference. So, this is very important here from NMR point of view.

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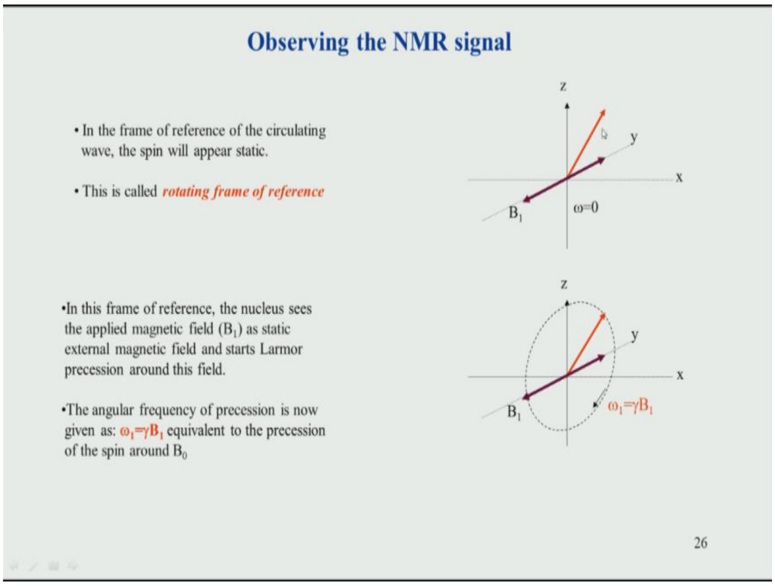

So, again look at this picture here, so this is our vector arrow which is our spin and this is what I showed you earlier is spinning around z axis. So, here the spin is not shown but you can imagine that this vector is moving around. Now you apply an RF radiation which again if you referred to the last slide we showed it is actually oscillating in the particular axis.

So, let us say it is going in the x y, y plane in oscillates like this back and forth. So now, if this oscillation that is oscillation which you are shown in the circular form is synchronized with this oscillation which is happening along z, and then we can think of it was it is called a rotating frame. A rotating frame of reference means you go into a frame in which the speed of the RF radiation or rotation of the RF radiation matches the frequency of RF radiation matches that of the spin.

So you can think of an analogy for example, in a train let a say there is a train which is moving at 100 kilometers per hour and you are standing outside the train. So, for you the train is moving at 100 kilometers per hour. But let us say there is a one more train parallel to that original train which is also moving at 100 kilometers per hour, so if you are sitting in the moving train the second train, for you the first train will be static because you are now at the same speed and therefore the other train is doses not move at all in your frame of reference.

So this is basically the same idea here that we go into the reference frame in which the frames are actually moving. So in that reference frame for we the spin will not move at all because now it is stack. I am also moving along with the spin at the same frequency. So, therefore this RF radiation which is applied, if it is synchronized with this particular spin rotation, then if I am if I am the RF radiation you can imagine like that if I am the RF for B the spins are no longer moving we are static okay.

So, therefore this is called rotating frame of reference a it is a frame of reference in which the spins no longer rotate because you have synchronized your rotation the RF radiation rotation with the spin rotation and that basically the spin appears, appears to be start it. So you have to keep in mind the spin is actually now static, but it appears to be static because we have now entered a different frame of references like a virtual frame in which the spins are static.

So, now this spins what will happen now is when the spins are static in the rotating frame what does it imply? It implies that now the spins are 0 frequencies because sorry spins are not precessing at all. Why are the spins are not precessing, because they are no longer they are synchronized with the magnetic field with the rotating field rotating frame and therefore the spins are 0. So, when you say the spin omega is 0 what it means. It means that now the external magnetic field itself has disappeared because remember omega = gamma into B0. So, when omega is 0 it basically indirectly implies that B0 is gone.

So, what essentially as happened now if you think of it carefully, what we have done is by going into a rotating frame of reference we have actually removed virtually the external magnetic field B0 and only thing which remains now is this particular oscillating B1. So this is very important concept which has to be very carefully thought about and you should I would recommended you to read through the books and understand this concept because is a very hard (())(11:13) to NMR is this concept.

So, the again I repeat is there what is happening is in the rotating frame of reference we have essentially removed the in external magnetic field. Remember it is not removed physically it is still there but in this frame of reference we have removed it, because that the omega is now 0, so, we can qualitatively think of that the B0 is gone to 0 and now only think what remains is B1. This is also a magnetic field because remember I mentioned in the last slide that the radiation which is applied is actually the magnetic field component of the electromagnetic radiation.

So, now the spins only look at B1 because they have no longer that B0 no longer appears to be there, so when they starts seeing the B1 and they start moving around the B1okay. So, they start now moving around the B1 because the B0 is gone and B1 is the what is only remains, so, they starts moving around B1. So, remember again the spins cannot differentiate between

B0 and B1, for them everything is a magnetic field, whatever is the magnetic field they start moving around that magnetic field.

So, in the first case when there was no B1 it was B0 but by going into rotating frame we have removed the effect of B0 and we are now seeing only the B1, so this means in the rotating frame. Remember which is again in the rotating frame in thus in the laboratory frame, that is in when you are outside this frame. In the laboratory frame, the spins are seeing both B0 and B1, but when you go through the rotating frame you are essentially the spins are only going looking at B1 and they now start moving around this B 1.

So, this is like a merry go round, so they actually precesses around the B1, which is basically remember the B1 is along the y axis. If B1 is along y axis then the spins will go from z to go back to - z and along in the z-x plane, so they are actually going from z to x to - z and so on, so it is like a merry go round this spins are now moving along the B1.

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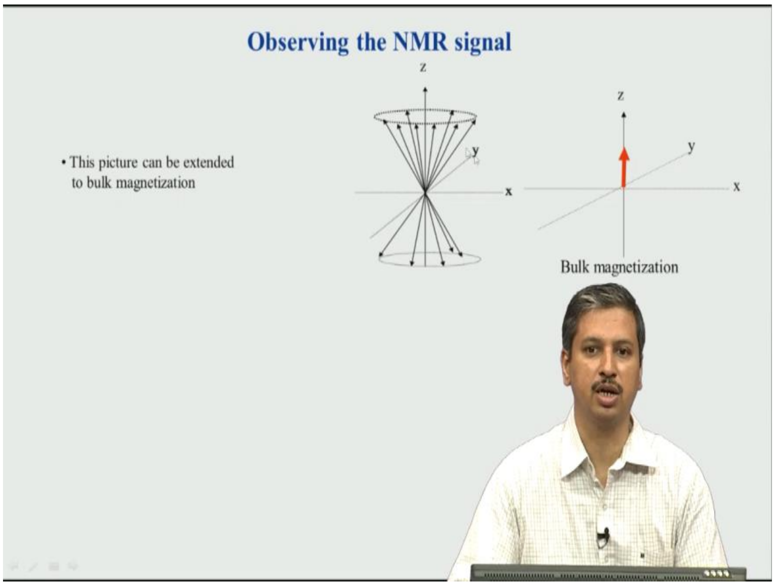

Now so this is what happens into the bulk magnetization. So, this is what I showed you earlier were essentially the rotation of the individual spins okay. So, now when you sum up all the spins together, this is what we saw that you end up with a small net vector which is pointing in the z direction. So, now our RF field now B1 radiation is applied along the y axis. So, when the B1 is applied along the y axis in the rotating frame the z component the z magnetization now will tilt towards x axis, so this is what we will see now.

So if you look at this bulk magnetization which is along z axis I apply an RF irradiation along y axis okay with from frequency and now in the rotating frame the external magnetic field is gone, so it now has to only left with B1. So, this red color vector will start moving around the B1 like a merry go around and it will go from z to x to - z to - x and so on, so this is what is shown in this small animation here. So, you see that the vector has come to this it will go down it will go less and it will go back. So this is what is called the B1, so what typically is done is that this B1 magnetic field is applied for a very short duration, then this typical duration is micro second and because of this very short duration in NMR we use the word pulse.

RF pulse is what is applied, so this is basically what it means, RF pulse is essentially a very short burst of energy which is given to the spins okay. So and the spins now for the very short duration start moving around the B1 field. So this is why we say is an RF pulse and RF pulse is a short burst of RF irradiation applied for a very short time that is typically micro seconds and then it is switched off. So, a short RF pulse can now this is at a more important point, the point is I will come back to this in the later slides, the idea here is that an pulse is so strong that it can excite all the spins in the molecule irrespective of their chemical shifts. So, when we come to this concept of chemical shift in NMR, where we will look at it more carefully this concept of uniform excitation, but right now the point to keep in mind is that what is done is to excite the molecules from a ground state to excited energy state. We have to do what cause what is called resonance.

So, the resonance is essentially a B1 field which is applied with the same frequency as the frequency of the spins, so it is gets synchronized an in the rotating frame the molecule starts spinning the spins start moving around the B1 field and that is applied for a short duration of micro second okay. So, this RF field we will look at this or again this point later that, this usually I applied at the center of the spectrum. So, what we will do this particular concept of frequencies and chemical shift we will come back to it a later point. So, let us move and see what this RF pulse causes.

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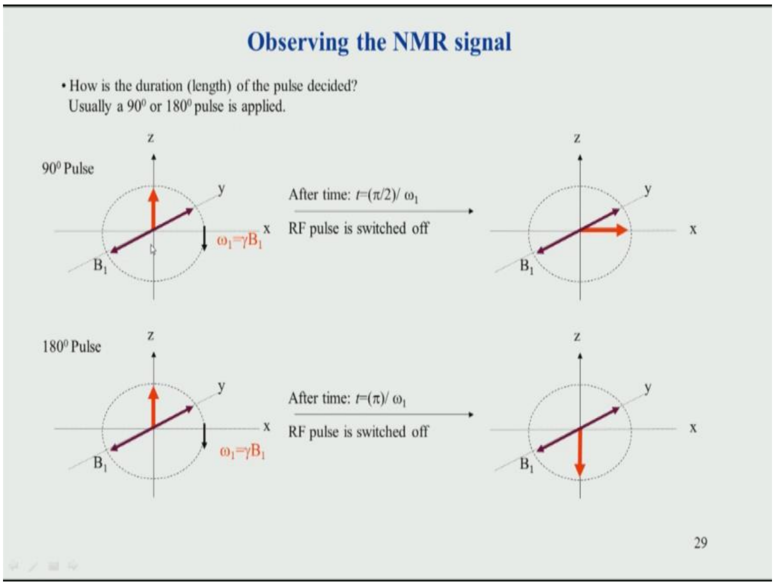

So this is again the same picture which you has showed in the previous slide. So, what is happening here is that you have applied a B1 field okay and to then magnetization which is along z axis, so this is called the equilibrium situation. This is basically the situation under which the system has not been disturb it is under equilibrium. But the moment we apply these B1 field a perpendicular, so this is the very important point here we have to keep in mind then this is perpendicular to the z magnetization and when we apply the field, it starts moving around this B1 field and after sometime it would have come to about 90 degrees.

So, this is basically a motion which is happening continuously, so it is not that it is happening in a very descript manner it is continuously going round and around like a around the B1 again, analogous to what I said merry go round. So, this is like a merry go around is going round and round, if you start from the first vertical position it has basically come 2 by 90 degrees to a to the x direction.

Now if I go further few more duration of this pulse that is B1 it goes to - z axis. So this particular position is called 90 degrees and if I stop the pulse now at this stage the spins would be now remaining along this, they will not go further because the B1 will be gone. So essentially we apply B1 field for a very short duration such that the magnetic magnetization that is red color magnetization goes from z and comes by 90 degrees to x, so this is called a 90 degree pulse.

After that magnetization has to come by 90 degrees, we remove this B1 and what happens after that we will see a little bit later, but this is typically that concept of a 90 degree pulse, which you will very routinely often see in all NMR experiment and this is a very important the 1D is a standard 1D NMR experiment is in fact nothing but a 90 degree pulse. So one should understand what is a 90 degree pulse and the 90 degree pulse is basically a duration that I is a that B1 field is applied for a very short duration such that during that time the magnetization comes from positive z axis to the x direction.

It can be this direction this direction all depends on where we apply the magnetic field, but the point here is that it has come by 90 degrees, so this is called a 90 degree RF pulse. And again remember it is called pulse because it is applied a very short duration. Now I can I do not, in some experiments I may want to go further I may not want to stop at 90 degrees. So, what I will do is again I will start from the beginning, I will take this a this the vector which is along z axis and apply a B1 field but this time the duration is not as pie by 2 that is 90 degrees it is double the time.

So if I double the time of this duration of the pulse, then the magnetization we will go further down and it would come by exactly 180 degrees, so this is called a 180 degree pulse. So in NMR, a 99% or 90% of the experiments essentially deal with either a 90 degree pulse or a 180 degree pulse, very rarely we will see a different angle flip pie, so this is called a flip angle. So what is typically when we use the in the one jargon of NMR or in the nomenclature we use the word the flip angle.

So, flip angle is basically the time or flip angle is angle by which the magnetic vector rotates around the B1. So, if it rotates by 90 degree, when the B1 is applied we say it is a 90 degree flip angle, if it rotates by 180 degrees we call it as a 180 flip angle. So, these two types of pulses the 90 degree pulse and 180 are basically the most important pulses and this will be the one which will be used always in all the experiments. Now let us look at what happens, when I remove the pulse.

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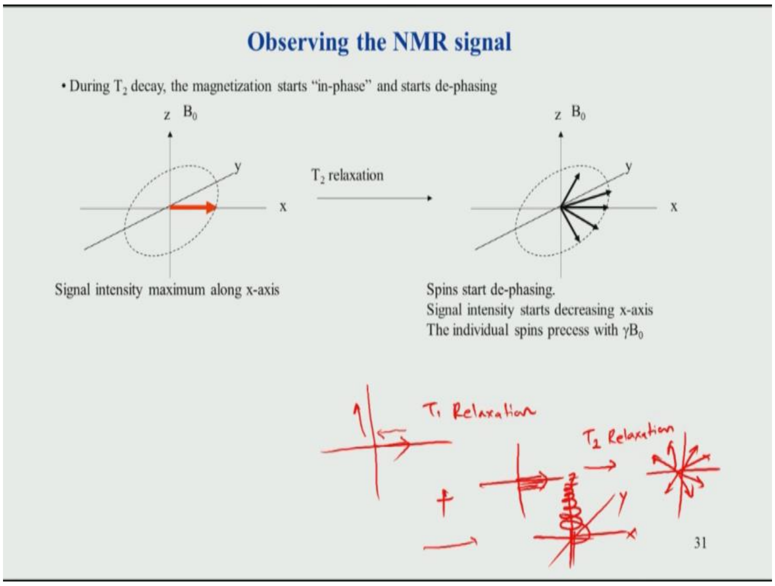

So, this is what is shown here, so this is basically again the magnetization has come by 90 degree. Now what I am going to do is I am going to remove this pulse that means the B1 is gone, so if you see in this picture it has gone, so should happen, what will happen is now the movement B1 is gone we are out of this B1 rotating frame in the sense we are out of the B1 field, so the magnetic field goes back towards its original position that is along B0, so this is called Relaxation.

So, relaxation is basically a phenomenon in which the magnetic magnetization vector returns to it is original position that is original situation, where it is pointing along the z axis and this happens only when you remove the B1 because as long as the B1 is present the magnetization keeps on rotating in the x-y plane, so in the z-x plane, only when you remove the B1 it has no effect of, it has to go back to the original effect, original situation and that is basically called relaxation.

So this is a very important concept NMR, in NMR there are two things which, the first one is called the chemical shift which we will see later and second is relaxation which is what us shown here. So, relaxation is basically the return the return of the magnetization to z axis. So, now when you see what is happened is, when the magnetization comes here, when you apply the B1 the initially the all the magnetization vectors, if you recollect the slides we showed, all the magnetization vector what distributed in a cone but when you apply an RF field they actually bunch together and they this is called a coherence.

So, what we have done is by applying a B1 field we have created coherence. The coherence is basically all of them having a same phase, but when you realize the B, when you remove the B1 they have to go back to the original random phase, remember the phase random phase approximation which was introduced and that says that original when it original situation when it goes back to the z axis, all the spins should be find out randomly along the z axis or they should be distributed along the z axis.

So, that process of going from a coherence state and this is a coherent state to a decoherent state we use the word T 2 relaxation. So T 2 relaxation is the word term used you will see often in NMR and will be very useful when we analyze the 1D NMR spectrum. T 2 relaxation is the relaxation by which the molecules the spins go back to the original condition where they are spin spend in the x-y plane in the random orientation. And the second point is there is another relaxation, so of you look at this vector again what is happening here is, this vector is now the magnetization is along the x-y plane in the x-y plane, but that is not is the equilibrium situation.

The magnetization needs to go back to z axis, so this process by which the magnetization goes from the x to z is called T 1 relaxation okay. So there are 2 different type of relaxation which are happening at the same time, but there are 2 different things for example, the first thing is as a said it is one is called decoherence, which is the T 2 relaxation and which the magnetic magnetization which is all coherent this start dephasing, so remember the word dephasing this start decoherence will look at it again it will come in many other aspects in start decoherence (())(23:34) dephasing and that is called T2 relaxation. And the process in which the magnetic the vector shrinks it starts shrinking in this direction and it starts building up in this direction that process is called T 1 relaxation.

So, if you visualize in your mind the what is this comes combine effect of these two, you can think of it like a spiral that the spins are actually spiraling they are going in a spiral form and they are slowly spiraling and then coming back to the z axis. So, this is what I can draw a picture here, so what is happening is the magnetic there are the spins are essentially that is like this, this is a vector so there are two things the magnetic the magnetization has to come this direction and it has to build up in this direction, this is called T 1 relaxation. And the other phenomenon is T2 in which all the vectors are pointing in this direction and then they have to start dephasing that means they have to start dephasing, they has to start dephasing and this is called T2 relaxation. So if you combine these two together, this T1 and T2 relaxation, what essentially you can visualize that the spins are actually going in in a spiral form.

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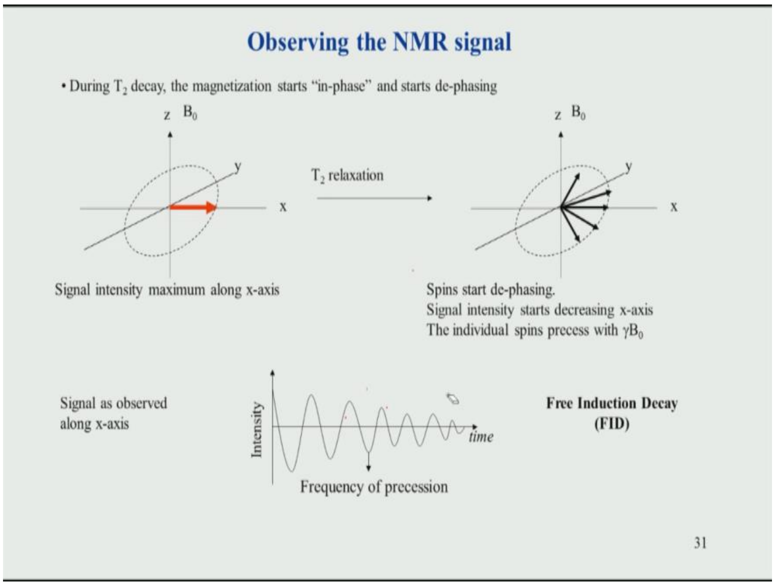

So this is what is happening these rotation this is x axis, this is z axis and I can draw a y axis here. So, what is happening in the x y plane they are rotating and slowly back coming back to z axis, this is a combination of T1 and T2. So, this is what happens when we look at T1 and T2 relaxation. So, let us now look at what happens in the relaxation process. So, during the relaxation process what is happening is the spins have to now this is what is shown in the spins now start dephasing this is a what I had drawn before and this is what is happening in the relaxation process.

And during this process, when they are actually fanning out, they are fanning out in the x-y plane they are each one of them is basically as individual spin, by if you look imagine these are different spins which are all rotating together and if there is a very famous if a principle of induction, it says that any magnetic this where any magnet which is rotating is induces a current in the coil, so this is the faradays law of induction. So, the magnets this, these are all tiny magnets, so when they are actually rotating in the x y plane they induced, so if you keep a coil let us say we keep at the coil here along x axis, when you keep a coil along x axis, these rotating magnets basically there all magnet which are rotating now is induced a EMF, EMF in this voltage in the coil which will be along let us say x axis.

So, that is what is shown here, that in there is a induced current there is a induced EMF in the coil which is kept along the x axis and this current you see here is oscillating, and why is this oscillating? The oscillation is coming because the spins are actually oscillating in the x-y plane. So the initially they are all bunch together there are all bunch together here red color there are all bunch together, so you get a maximum signal because there all pointing in the same direction, but as this spins start moving around I mean dephasing then they are start the the vector along x axis which is all here shown here starts losing it is amplitude, because the spins are now dephasing in the x-y plane.

So, this loss of amplitude is now depicted here in the form of a oscillation. So, these vectors all of them go to the negative axis, they will go negative, then they will come back to positive and slowly, when the everything is all of them have dephased completely, the emf which is induced is completely gone to 0. So, this is basically the idea of what is called as a free induction decay, so very important concept in NMR we use the word FID. So, a free induction decay is nothing but the induction of a current in a coil as, as a spin start relaxing back after the RF pulse is removed.

So this is the signal which is actually captured or detected by a coil which is sitting a let us say along x or y direction, any direction is it is okay and that direct that particular coil capture this signal and that signal is decaying slowly to 0 and that is because of the rotation of the spins along the x-y plane. And this is going to be our signal and this is what is going to give us a NMR spectrum which we will see in the next class.