# NMR spectroscopy for Structural Biology NS
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## Lecture: 04
## NMR Basic Concepts – IV

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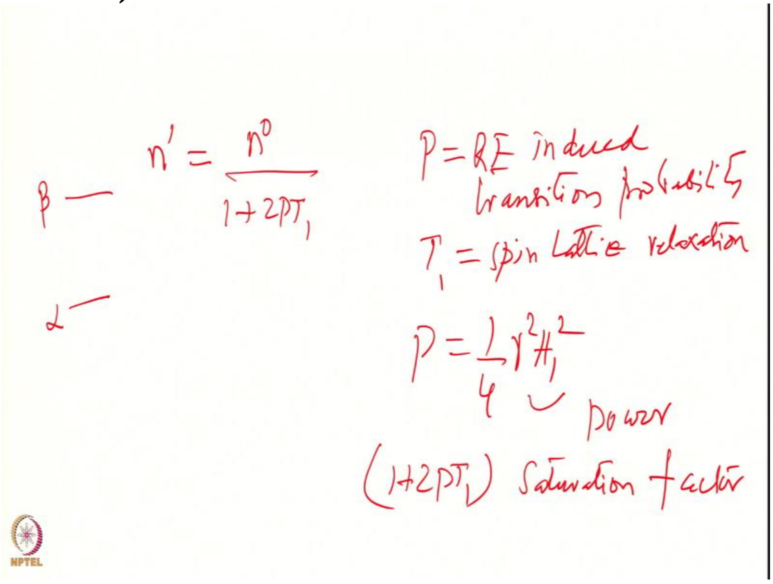

β —

α —

$n' = \frac{n^0}{1+2PT_1}$

P = RF induced transition probability

$T_1$ = spin lattice relaxation

$P = \frac{1}{4}\gamma^2 H_1^2$

power

$(1+2PT_1)$ saturation factor

So, we talked last time about the absorption of energy by the spin system when we apply an RF. So, considering the relaxation also we said there is a steady state population difference between 2 energy levels. 2 energy levels we are considering for a spin ½ system α and β and this n’ is the steady state population difference between the 2 and that is given by

$$n' = \frac{n_0}{1 + 2pT_1}$$

$n_0$ naught divided by 1 + 2PT 1 and where P is the RF induced transition probability and $T_1$ is the spin lattice relaxation time.

Is because of this spin lattice relaxation while the RF tends to reduce the population difference the $T_1$ tends to restore this population difference therefore as a result of the balance between these 2 we have a continuously non-zero population difference. And therefore we get the NMR signal we also said $p = \frac{1}{4}\gamma^2 H_1^2$

P is equal to 1 by 4 $\gamma^2$ $H_1^2$ where $H_1$ is the amplitude of the RF and γ is the gyromagnetic ratio.

So, in $H_1^2$ this defines the power while $H_1$ is the amplitude $H_1^2$ defines the power of the RF. How much power you apply the more the power higher is the transition probability you tend to reach the saturation. So, therefore this $1 + 2pT_1$ is this term is called as the saturation factor because if this is too high if the $1 + 2pT_1$ is very, very large then of course n’ may go down to 0 in which case of course you will lose the signal that is why this is called as the saturation factor $1 + 2pT_1$.

If which is very, very large then you have the signal disappearing and therefore this is called as the saturation factor. So, now from here we go forward and to see now next we ask what kind of transitions the RF will induce.

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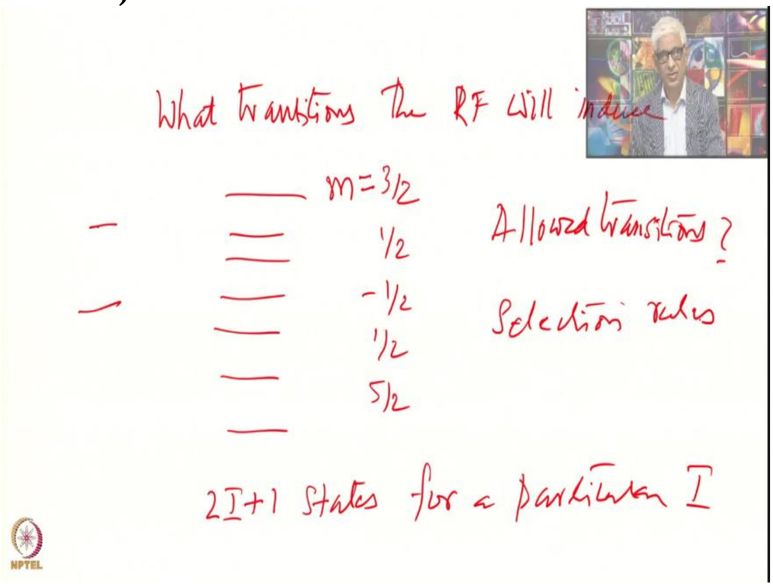

What transitions the RF will induce

m = 3/2

1/2

-1/2

1/2

5/2

Allowed transitions?

Selection rules

2I+1 States for a particular I

So, what transitions the RF will induce in other words suppose we have a spin system which has many energy levels. So, far we are considering only 2 energy levels. So, therefore there is no issue but suppose I have a system which are many levels like this and this will depend upon the value of I because we said if the there are 2I + 1 states for a particular I that is for considering if there is a one spin with one type of spin which has the I value that 2I + 1 states.

If there were multiple spins then of course you will have a net I value and which will of course be the very different and you will have many different orientations possible many different states possible for the combination of this of the various states. What sort of transitions are possible here. So, these are various values of m here if I call it as m what is the magnetic quantum number.

So, l let us say this has a $\frac{3}{2}, \frac{1}{2}, -\frac{1}{2}, or\ \frac{5}{2}$ and so on and so forth. We have various kinds of spin sets which are possible what sort of a transitions are allowed. So, in other words what are the allowed transitions? The same thing is to say what are the selection rules. Does the RF induce transition between every 2 levels? So, it turns out that this comes out from quantum mechanics by the perturbation theory in quantum mechanics.

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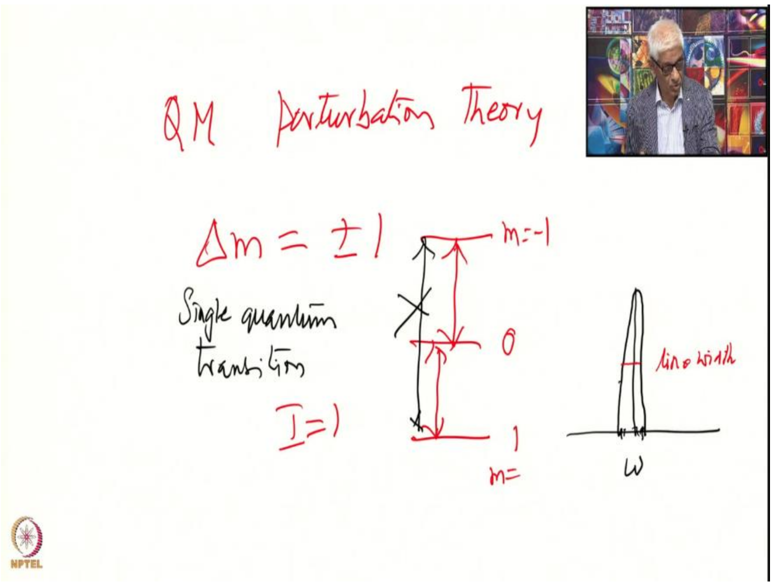

Quantum mechanics perturbation theory will not go into that detail perturbation theory it turns out that RF can induce only those transitions for which delta M is equal to plus minus 1. So, it cannot reduce transition between m is equal to if I have a let us say for $I = 1$ what are the states possible? I will have $I = 1$ $m = 1$ this is $m = 1$ and then you have the 0 here and you also have $m = -1$.

So, $\Delta m = -1$ meaning what states are possible? We can have a transition between these 2 levels we can have a transition between these 2 levels. So, these are both $\Delta m = \pm 1$. So, but this transition this transition is not allowed this will not be allowed because that black one $\Delta m = 2$ and that is not allowed and that is typically known as a double quantum transition.

Wherever there is $\Delta m = -1$ this is called as single quantum transition. So, this is so far as the energy absorption is concerned. Now it leads us to the next question will the energy absorption happen precisely only at that frequency or there is a certain variation allowed. So, is the NMR spectrum if absorption of energy happens exactly at one frequency if this is $\omega$ does it happen exactly at one frequency or some other frequencies which are nearby?

Will also cause a transition will also cause absorption of energy what does that mean if I have a certain kind of possibility of having a transition for these energy levels also they may have different intensities all right then I will get a profile of line like this. So, the frequency here if I apply an RF frequency here it will have an amplitude absorption of energy will be this much if this exactly a resonance condition it will have a highest amplitude.

If it is frequency is here then I will get this much of amplitude there and therefore it will generate portion of energy different RF excitation f efficiency will be different. This width here at half height this width here at half height this is called as the line width. So, the absorption of energy does not occur only at one frequency but there is a certain tolerance. So, it there is a certain tolerance on what should be the for the frequency for absorption to take place this is called as the line width.

Now what does this line weight depend upon. So, this is something which one has to see we can look at that.

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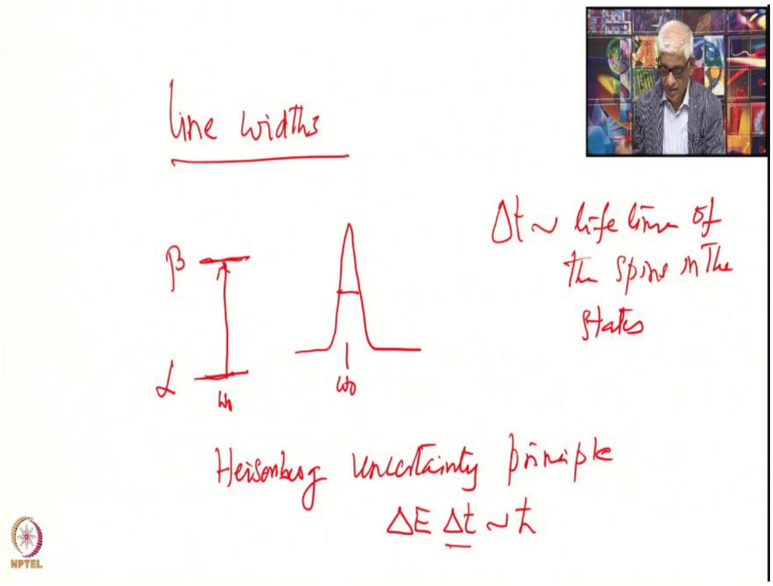

line widths

$\Delta t \sim$ life time of the spins in the states

Heisenberg uncertainty principle

$\Delta E \, \underline{\Delta t} \sim \hbar$

So, the line widths what are the factors with influence the line widths. Now consider let us say a 2 level system. So, we have the exact frequency one value but there are certain other values we said it will have absorption of energy possible and we are going to observe a signal which is looking like this and this is the central value omega naught and I will have this width which is here what does it depend upon.

Basically this comes from a principle known as Heisenberg uncertainty principle. What it says is in quantum mechanics in certain in particles of this type there is a definite uncertainty in the energy values and there is a certain uncertainty in the time if there is a certain uncertainty this product is approximately equal to h cross. Uncertainty in the energy there is an uncertainty in the time.

Now what is the meaning of uncertainty in time uncertainty in time means this is the lifetime of the state. So, we can take this uncertainty in time to imply lifetime of the states of the spins in the states. So, what does that mean if a particular spin is in the particular state. Let us say this is α and this is β will it remain there forever, no it does not remain there forever there is always a certain continuous transitions going on from up and down α to β and β to α these transitions are continuously going on.

These are induced by the lattice or induced by the RF or whatever. So, there is a kind of a lifetime for the spin in a particular state and that is called as the lifetime. And therefore if that is Δt accordingly if this condition has to be satisfied there is a certain uncertainty in the ΔE also. Therefore that uncertainty reflects in this width when there is an uncertainty in the energy level.

So, it is either here or here or here or here some uncertainty in the energy value. Then it will cause absorption of energy at slightly different frequencies and that is what is called as the line width. Now therefore whatever affects the lifetime of the individual states that will cause a line width.

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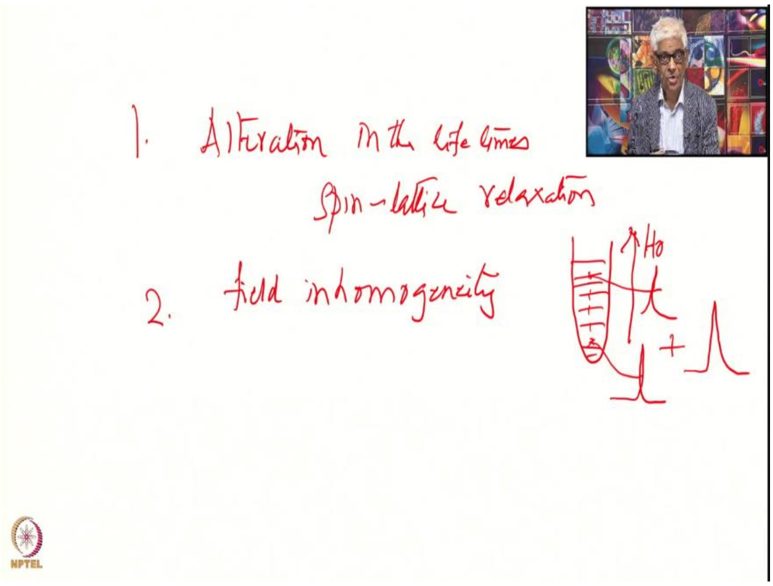

1. Alteration in the life times

Spin-lattice relaxation

2. field inhomogeneity

So, therefore alteration in the line width, so one reason is alteration in the in the lifetimes we will not go into the details as to what all these things can happen of course this is reflected in what we already seen in the spin lattice relaxation. We have seen this in the spin lattice relaxation this will alter the line width and the phase coherence changes that is that we will see afterwards. And then there is a second reason what can cause line width this is the field inhomogeneity.

What is the meaning of that? Suppose I have a sample like this I have the solution here the various spins are present at various places here now apply I am applying a field which is H naught suppose this field keeps fluctuating the value of a particular spin of a value of field at this point is slightly different from the value of the magnetic field at this point then this fellow will absorb energy at one frequency this fellow will absorb at another frequency therefore the result will be.

So, this may give you a free an absorption at this position and this may give me an absorption at this position these are slightly changed slightly different this plus this will give broader line. So, therefore this contributes to the line width because both the things are present the same spin absorbs energy at 2 different frequencies because of the field inhomogeneity.

So, this is a different mechanism earlier one was the lifetime of the individual states has changed and this is an in different mechanism this can be controlled by adjusting the homogeneity of the sample very well homogeneity of our magnet very well this can be controlled. So, this is an important contributor to the line widths. There are various other reasons why there can be changes in the lifetimes.

And these are all based on the quantum mechanical principles it is sufficient to know whatever alters the lifetimes of the individual states that will cost contribute to the line width. So, now let us look at the description of the spin system from a different viewpoint and this is how Felix Bloch described this magnetic resonance phenomenon in a classical way in a phenomenological way and he set up a set of equations called as Bloch equations.

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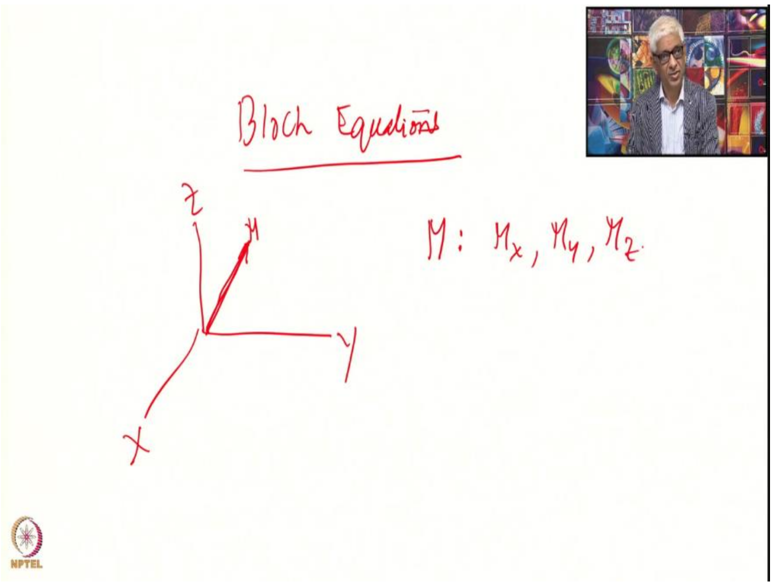

These are known as block equations. So, he described the moment of the magnetization in the presence of the RF how it will in a very generalized manner. Let us say we have the axis like this here x y z and we have a generalized representation of the magnetization which is let us say oriented somewhere like this. This is my magnetization M. Now this is the vector the magnetization is a vector right.

So, the magnetization will is vector means I have components $M_x$, $M_y$ and $M_z$ these are the components along the three axis.

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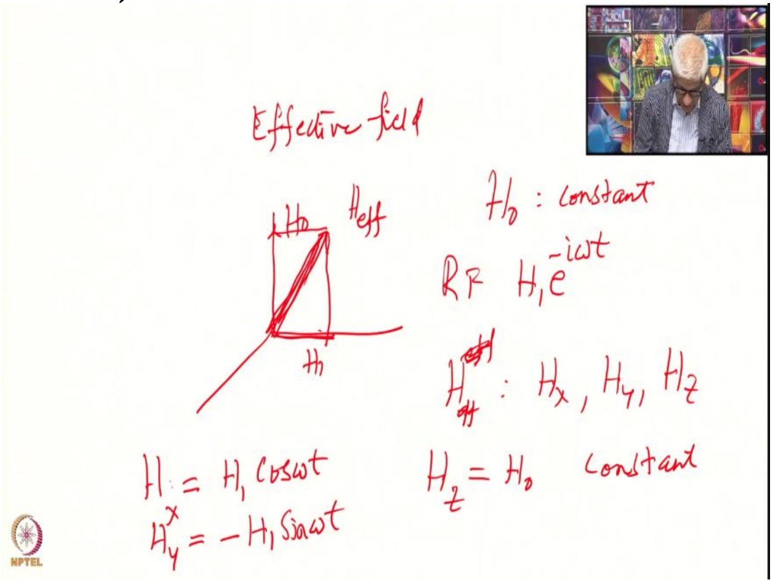

There is also this effective field and that is like this. So, I have this $H_0$ field along this axis this is the main field then I have applied a RF which is the $H_1$ along this axis let us say then therefore the effective field is the vector addition of these 2 fields I will get a field $H^{eff}$ (effective). So, if the $H^{effective}$ is oriented in this manner of course since RF is a rotating field this h effective also keeps rotating h effective also keeps rotating.

So, therefore this has a time dependent component and there is a time independent component. Now $H_0$ is constant is constant and the RF is $H_1e^{-i\omega t}$ H1 e to the minus I omega t this is the time dependent component here and this is the rotating field therefore the h effective also keeps rotating. So, therefore it has these components let us represent this $H^{eff}$ with three components as now this is effective here $H^{eff}$ here this has the components $H_x$, $H_y$ and $H_z$ of these $H_z$ is constant $H_z = H_0$.

This is constant and $H_x$ we write it as $H_x = H_1 cos\omega t$ and $H_y = -H_1 sin\ \omega t$ because it is going in the $e^{-i\omega t}$ is what $os\omega t$ , $-i\ sin\ \omega t$ . Therefore so this $H_x$ remember this is goes to this one here $H_y$ is down there. So, therefore we have three components and these three components are we can write explicitly like this.

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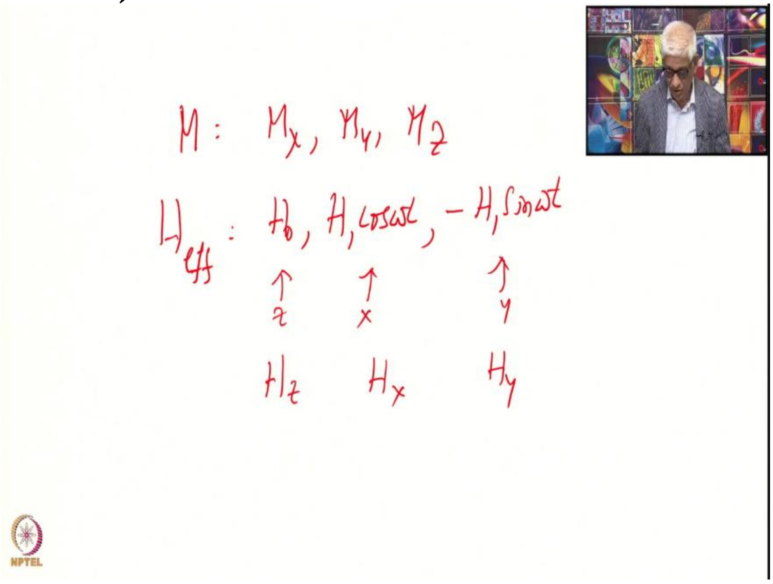

$$M : M_x, M_y, M_z$$

$$H_{eff} : H_0, H_1 \cos\omega t, -H_1 \sin\omega t$$

$$\begin{array}{ccc} \uparrow & \uparrow & \uparrow \\ z & x & y \\ H_z & H_x & H_y \end{array}$$

So, I have the M vector M vector has the components $M_x$, $M_y$, $M_z$ and the $H^{eff}: H_0, H_1 cos\omega t, -H_1 sin\omega t$. Now notice here this $H_0$ the Z axis here $M_x$ and $M_y$ or they are in different order this is along the Z axis this is along the z this is along the X this is along the Y $H_x$ $H_y$ I wrote here $H_z$ $H_x$ and $H_y$. So, let me write again down here $H_z$ $H_x$ $H_y$.

So, Bloch wrote a set of equations to describe the moment of the magnetization vector in the presence of such a kind of a $H^{eff}$.

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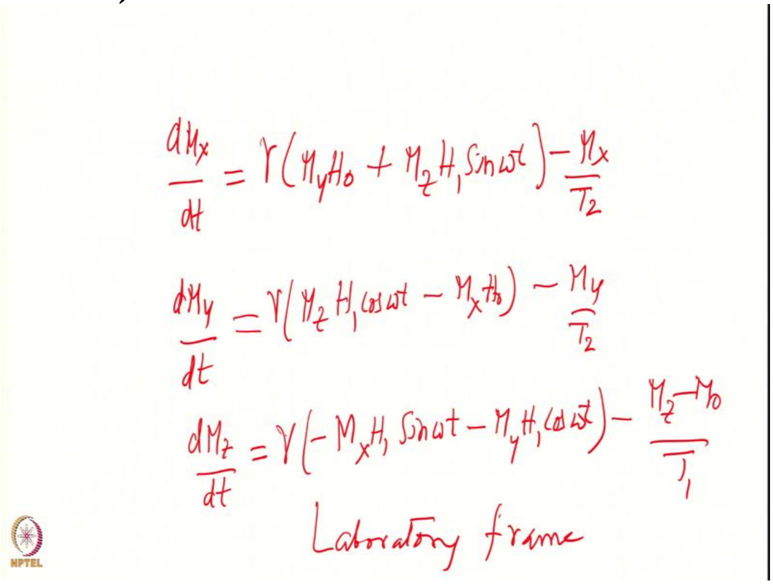

$$\frac{dM_x}{dt} = \gamma(M_y H_0 + M_z H_1 \sin\omega t) - \frac{M_x}{T_2}$$

$$\frac{dM_y}{dt} = \gamma(M_z H_1 \cos\omega t - M_x H_0) - \frac{M_y}{T_2}$$

$$\frac{dM_z}{dt} = \gamma(-M_x H_1 \sin\omega t - M_y H_1 \cos\omega t) - \frac{M_z - M_0}{T_1}$$

Laboratory frame

So, I will write those equations here directly and of course we also considered the relaxation phenomena. So, $\frac{dM_x}{dt} = \gamma\ (M_y H_0 + M_z H_1 \sin\omega t) - \frac{M_x}{T_2}$. Now $T_2$ is the relaxation time which I had introduced to you earlier. This is the spin-spin relaxation time or it is a transverse relaxation time $\frac{dM_y}{dt}$ this is the rate of change of the y component of the magnetization.

Because remember these are time dependent phenomena because of the RF which is time dependent and the relaxation is also it changes the magnetization in a time dependent manner therefore this these all of these equations will have time dependence. So, this is $\frac{dM_y}{dt} = \gamma\ (M_z H_1 \cos \omega t + M_x H_0) - \frac{M_y}{T_2}$. And these obviously these $M_x$ and the $M_y$ components are the transverse components.

Therefore they depend upon the transverse relaxation time $T_2$ now what about the z component $\frac{dM_y}{dt} = \ \gamma(-\ M_x H_1 \sin \omega t -\ M_y H_1 \cos \omega t) - \frac{M_z - M_0}{T_1}$. So, the first is the transverse components which are present here and the last relaxation part which is there this is dependent on the $T_1$ relaxation time.

$M_z$ is the magnetization at any point in time M, $M_0$ is the equilibrium magnetization. So, depending upon the deviation of $M_z$ from $M_0$ it will have a relaxation going on that the magnetization $M_z$ is going to change. So, this is these are the Bloch equations in the so called laboratory frame. But the NMR experiment is done actually in the rotating frame.

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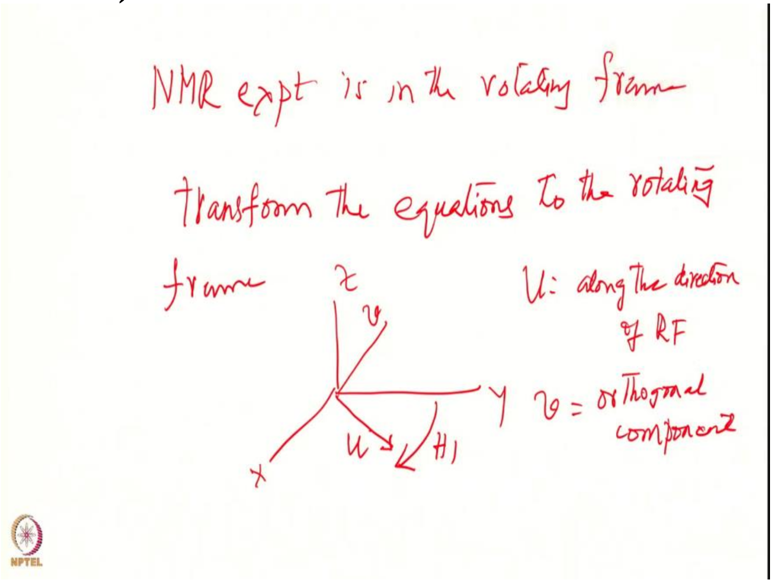

NMR expt is in the rotating frame

Transform the equations to the rotating frame

U: along the direction of RF

v = orthogonal component

NMR experiment is done in the rotating frame is in the rotating frame that is as if you are sitting on the RF and looking at the magnetization how it is spinning. So, therefore the main RF frequency will get subtracted out the time dependence will go away in the rotating frame meaning you sit on the RF and look at the magnetization in the rotating frame. Therefore you can transform the equations into the rotating frame.

I am not going to give the details of that one but I will only give you the results transform the equations to the rotating frame. Rotating frame is you have this the RF is applied the RF is moving here this is the $H_1$ and the component of $H_1$ which is let us say you are sitting on it and looking at this is the $H_1$ here and what is on that axis if the $H_1$ is rotating in this field I will have that particular axis I am sitting over here.

And there is also a the orthogonal component which is going like this it will have an orthogonal common this is the normal x y z components then I will have sit on the RF and look at it in the same direction suppose it is moving like this. So, I sit on it I have this axis here and then there is the other axis which is perpendicular to it. So, that perpendicular axis I am writing like this. So, I call this axis as u and this axis as v.

So, I measure the magnetization in these 2 in these magnetization components along the u and the v axis. So, therefore u is the magnetization component in the direction where the RF is applied and u is the orthogonal component. So, u is along the direction of the RF and v is the orthogonal component. So, this is the mathematical operation how to transform it. So, you have the magnetization M which you can write in the laboratory frame as $M_x$ $M_y$ $M_z$.

Or you can write in the rotating frame as u component v component and the z component which is $M_z$ only that component does not change and that is the u component. So, now I make this transformation after doing the transformation I will write the Bloch equations.

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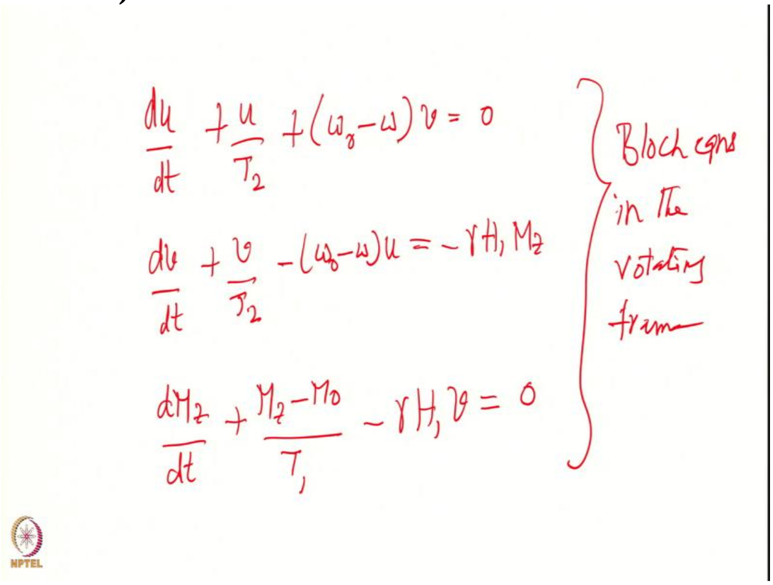

$$\frac{du}{dt} + \frac{u}{T_2} + (\omega_0 - \omega)v = 0$$

$$\frac{dv}{dt} + \frac{v}{T_2} - (\omega_0 - \omega)u = -\gamma H_1 M_z$$

$$\frac{dM_z}{dt} + \frac{M_z - M_0}{T_1} - \gamma H_1 v = 0$$

Bloch eqns in the rotating frame

And this will be $\frac{du}{dt}+\frac{u}{T_2}+(\omega_0-\omega)v=0$. Then I will write similar one for $\frac{dv}{dt}+\frac{v}{T_2}-(\omega_0-\omega)u=-\gamma H_1 M_z$ and for the $M_z$ this does not change because this is along the Z axis it is a constant term this does not depend upon the rotating frame $\frac{dM_z}{dt}+\frac{M_z-M_0}{T_1}--\gamma H_1 M_z=0$.

Now it is dependent on $T_1$ notice $\gamma H_1 M_z=0$. So, these are the three equations in the Bloch equations in the rotating frame.

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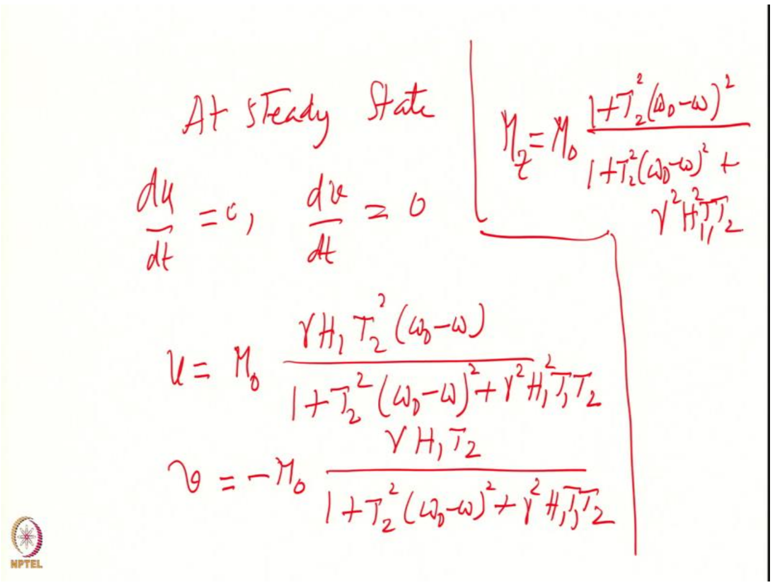

At steady state

$$\frac{du}{dt} = 0, \quad \frac{dv}{dt} = 0$$

$$u = M_0 \frac{\gamma H_1 T_2^2 (\omega_0 - \omega)}{1 + T_2^2 (\omega_0 - \omega)^2 + \gamma^2 H_1^2 T_1 T_2}$$

$$v = -M_0 \frac{\gamma H_1 T_2}{1 + T_2^2 (\omega_0 - \omega)^2 + \gamma^2 H_1^2 T_1 T_2}$$

$$M_z = M_0 \frac{1 + T_2^2 (\omega_0 - \omega)^2}{1 + T_2^2 (\omega_0 - \omega)^2 + \gamma^2 H_1^2 T_1 T_2}$$

So, now at steady state what happens at steady state as I said we are going to measure the signal continuously. So, therefore at steady state what will be the situation at steady state all the time derivatives will be 0 at steady state $\frac{du}{dt} = 0$ and $\frac{dv}{dt} = 0$. If you substitute these into those block equations then you will get $u = M_0 \frac{\gamma H_1 T_2^2 (\omega_0 - \omega)}{1 + T_2^2 (\omega_0 - \omega)^2 + \gamma^2 H_1^2 T_1 T_2}$.

And $v = -M_0 \frac{\gamma H_1 T_2}{1 + T_2^2 (\omega_0 - \omega)^2 + \gamma^2 H_1 T_1 T_2}$. And M$_z$ I will write the M$_z$ here. $M_z = M_0 \frac{1 + T_2^2 (\omega_0 - \omega)^2}{1 + T_2^2 (\omega_0 - \omega)^2 + \gamma^2 H_1^2 T_1 T_2}$.

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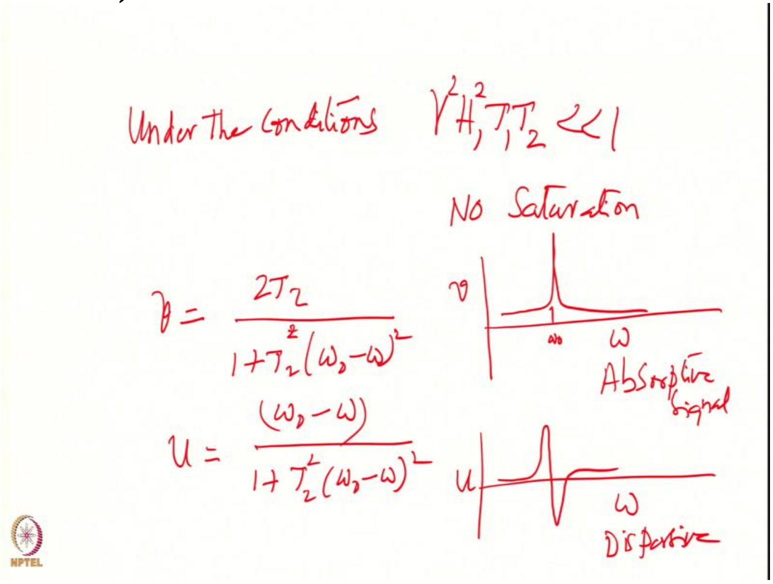

Under the conditions $\gamma^2 H_1^2 T_1 T_2 << 1$

No Saturation

$$v = \frac{2T_2}{1 + T_2^2 (\omega_0 - \omega)^2}$$

$$u = \frac{(\omega_0 - \omega)}{1 + T_2^2 (\omega_0 - \omega)^2}$$

Under the conditions $\gamma^2 H_1^2 T_1 T_2 \ll 1$ that is this represents no saturation remember saturation is dependent on the power H$_1$. So, H$_1$ pair $H_1^2$ is very small compared to the T$_1$ and the T$_2$ when you take the product is very small then you will get the simplified equations for $u$ and $v$ then you get $v = \frac{2T_1}{1 + T_2 (\omega_0 - \omega)^2}$.

And $u = \frac{(\omega_0 - \omega)}{1 + T_2^2 (\omega_0 - \omega)^2}$. These are the components which we are actually going to measure these are the transverse components we are going to measure this. Now if I want to draw this curve

I want to plot v versus omega. So, if I plot *v* versus ω this is ω here this is *v* here how does it look? This will look like this and this is $\omega_0$ center is $\omega_0$.

If I plot *u*, *u* versus ω how does this look? This will look like this down like this like this. So, this is called as the absorptive signal and this is the dispersive signal. So, these are the 2 basic line shapes in your NMR spectrum. So, but most of the people use this absorptive line shape because it is a very it does not have the positive negative components and it is sharp it does not have the tails as this u component has.

So, this is the measurement. So, therefore NMR signal, NMR spectra you will see this term this is what you will observe in your NMR spectra. So, I think we can stop here for this class and we can go over to the other things in the next class.