NMR Spectroscopy for Chemists and Biologists
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Lecture 15
Practical Aspects of Fourier Transform NMR Spectra

Let us continue with the discussion of the FTNMR spectrometer, we introduced in the last class. So just to have a recap I have this some summary of this FTNMR spectrometer in this slide.

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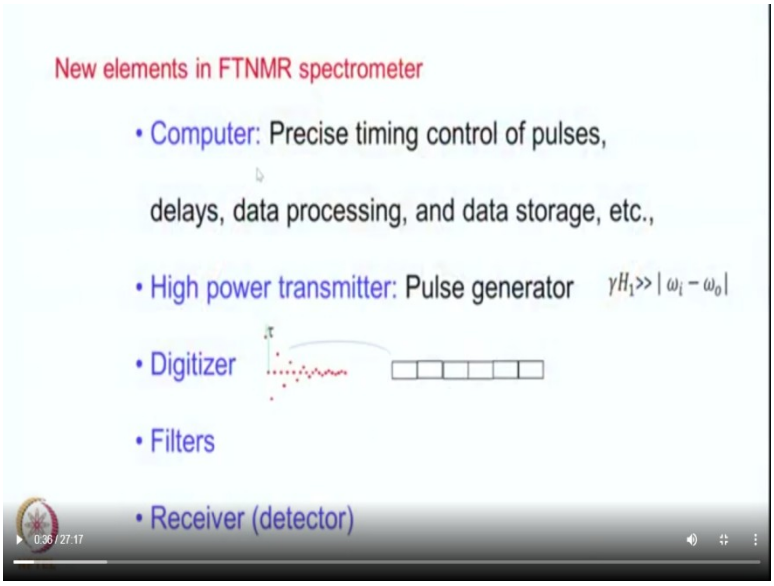

New elements in FTNMR spectrometer

- Computer: Precise timing control of pulses, delays, data processing, and data storage, etc.,
- High power transmitter: Pulse generator $\gamma H_1 >> |\omega_i - \omega_0|$
- Digitizer
- Filters
- Receiver (detector)

So that new elements in the FTNMR spectrometer, first thing is the computer because everything is computer controlled now. Now we have to be precise with respect to the timing of the pulses, the control of the pulses and the various other aspects of the pulses as we will see many times in due course and the delays have to be properly adjusted and to be controlled because we have to say we have to give a pulse of 10 point 2 microseconds and things like that.

And then the data processing is an integral part of the FTNMR spectrometer because there is a Fourier transformation, which is one of the important elements and there are many others related to that, then the data storage the storage is in the binary form in the computer. So lot of things keep added as a result of this change in the spectrometer structure. An important element which we also mentioned is the high power transmitter.

The pulse has to have high amplitude, the RF for the pulse has to be very high amplitude because we saw in order that the pulse is exciting a whole range of frequencies and the effective field is along the H_1 field which is the *RF* amplitude. The amplitude has to be much more than the entire spectral range $\omega_i - \omega_0$, ω_0 is the *RF* frequency ω_i is the frequency of a particular signal in your spectrum and for the whole range of I's, this condition has to be satisfied then only your effective field will be along the H_1.

Then you can rotate the entire magnetization about the axis of the *RF*. So that requires a very high power. This is unlike what is there in the slow passage experiments where this amplitude is very very small. Remember this $\gamma^2 H_1^2 T_1 . T_2$ has to be much large smaller than 1 for the saturation to be avoided but here that is not the case you excite all the spins in one go and that is achieved by using a very high power H_1. And then you have this signal which comes is actually an analog signal, the data you collect there you detect the y component or the *x* component of the magnetization, it is collected in a digitized manner.

The signal comes in analog fashion, all right it is in FID the free induction decay is a continuous signal. However, if it is stored in the computer, it has to be stored in the digitized manner. So we digitize the signal and collect data points at regular intervals of time, say we have time between two points is $\frac{1}{\tau}$ and each of these τ points is put in the in a digitized form and here is the data which is put in the digitized form and this is in the binary form, so we have various bits here we say this is called as the analog to digital converter and it has sort of a certain number of bits and it is stored in each of these bits.

So you can for example you can have 1 0 1 0 1 0 or 1 1 1 1 1 that is your the binary points and this is called the analog to digital converter. In briefly it is also called as ADC and once the data is converted into the digitized form, it is put in the computer in as a word each point here goes as a word in the computer.

And when you add the word for signal averaging then of course, each of these points gets added again and again and again signal averaging happens in the computer. Then you have filters, the filters we will see in the next slide and filters are used to filter out unwanted spectral excitations and detection systems and the receiver is the one which actually collects the data and we have phase sensitive detectors, which collect either the x component of the magnetization or the y component of the mechanization.

Okay, now let us turn to some practical aspects of FTNMR spectra. As we said earlier, the pulse, pulse excitation is

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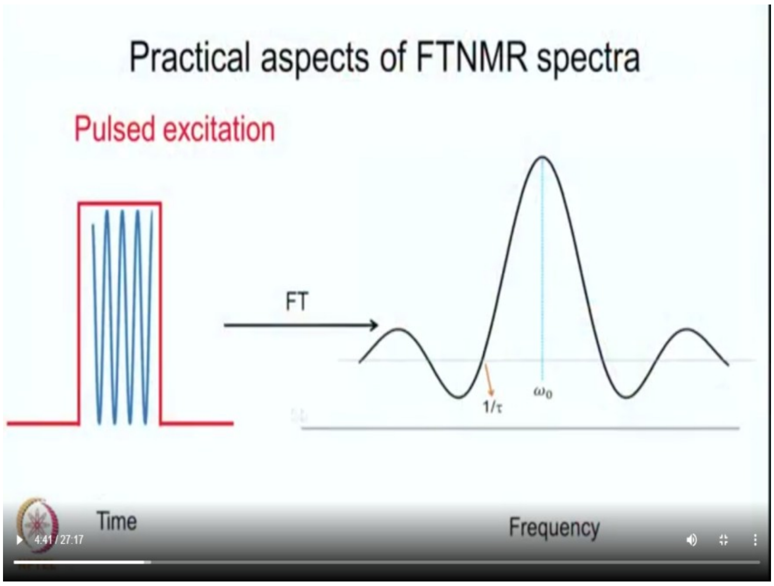

Practical aspects of FTNMR spectra

Pulsed excitation

The generations of whole lot of frequencies in one go and this is achieved by applying the RF for a very short period of time and this is of the order of microseconds, few microseconds, if the width of this is tau then we will say it will excite the frequencies in this particular fashion, this is excitation profile of the RF pulse, these are Fourier-related.

So whole range of frequencies are excited with a different amplitudes and omega not is the RF frequency and at this point it is 1 by tau 1 by tau is if the tau is of the order of 1 micro second, this will be 1 megahertz, okay megahertz here and a megahertz here, so this covers a whole range of megahertz, but however, all of these will have different amplitudes and therefore a different powers.

However, we do not want that sort of a thing, we need to have excitation, uniform excitation over the spectral range. Suppose your spectral range is only a small region of some 10 kilohertz or something like that which will be just a region around this and that for example, we indicate here if this is the region which is region of excitation region of your interest and here it is $\omega_0$this is my ω_0.

So this is the frequency which is the *RF* and around that *RF* we take a small region here and this small region which will have similar amplitudes because if I want to excite the various spins to the same level then I must have the same power applied to all of them. Therefore, we choose a small area here and this is achieved by what are called as the filters which are mentioned in the previous line.

Okay, the other electronic devices which allow you to filter out all the other signals which are excited by the application of the *RF* and we pick out only this much area. So excitation at any frequency ω_i is proportional to the square of the amplitude of or and that is called as the power here is the amplitude which is plotted but the square of that is the power. So the pulse excitation profile is to be tuned to the region of your spectral width or the way you spectra how much spectral width you want accordingly one has to tune their filters.

Okay, now we know that excitation happens to a certain region around the spectral width, right?

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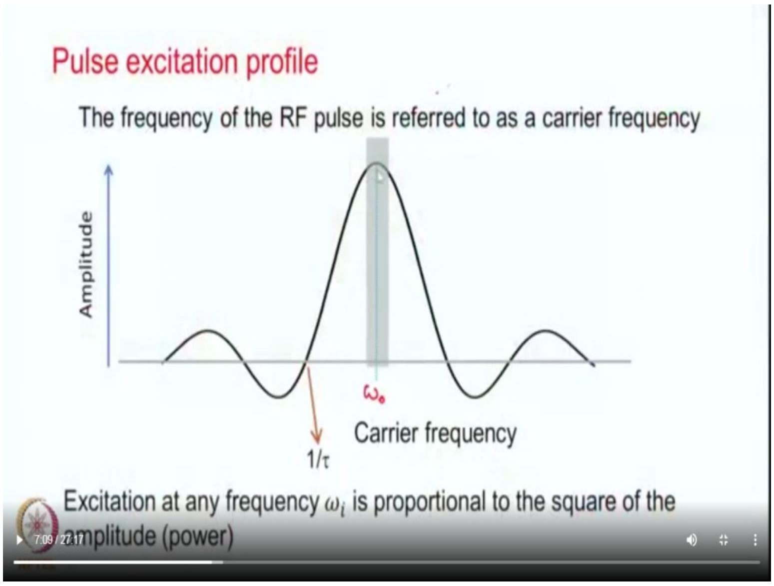

Pulse excitation profile

The frequency of the RF pulse is referred to as a carrier frequency

Excitation at any frequency ω_i is proportional to the square of the amplitude (power)

So we said that this much area is area which you want to choose. Now suppose you have a carrier frequency which is here, but your signal of interest lies is somewhere here. Then what happens, this signal this pin is not excited properly why this excitation pulse? So therefore what one has to do, we will have to actually shift this omega not to this place. Otherwise, what is the consequence and that we will see in the next slide here.

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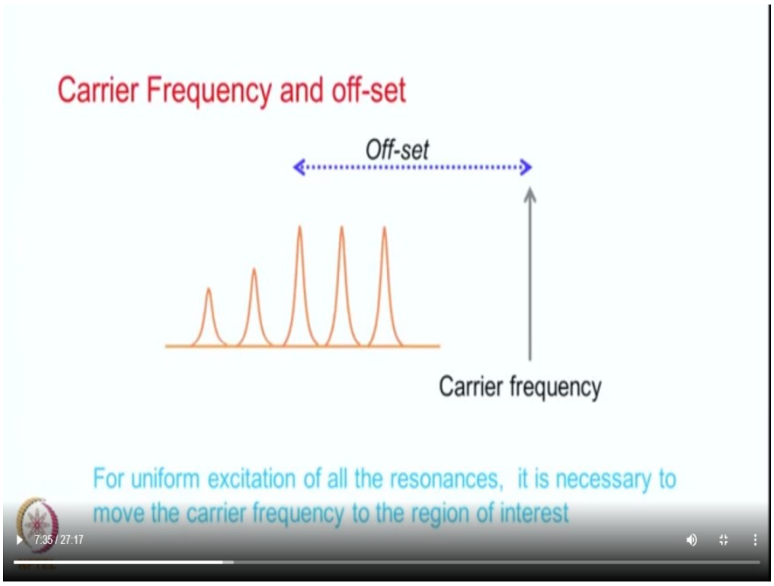
Carrier Frequency and off-set

For uniform excitation of all the resonances, it is necessary to move the carrier frequency to the region of interest

You see here, if your carrier frequency, which is your *RF* frequency put in a particular place this is omega not frequency here and if you put it here, which is far away from the region of your interest. That is then what happens is some signals are excited with this intensity, some signals are excited with the lower intensity because the *RF* power is low here.

So we do not want that thing to happen we want to have a uniform acceleration of all these spins and therefore we will have to shift this carrier frequency to the center of this origin of the spectrum, and this is what you do. So once you shift this carrier frequency from there to here then you will see the whole range is covered properly and all the spins are excited to a similar extent and you get similar intensities for all the signals and this is crucial for any quantitative evaluations of your or samples or molecules the signals are to be excited to the same extent. So for uniform excitation of all the resonances it is necessary to move the carrier frequency to the region of interest and this is called as the offset.

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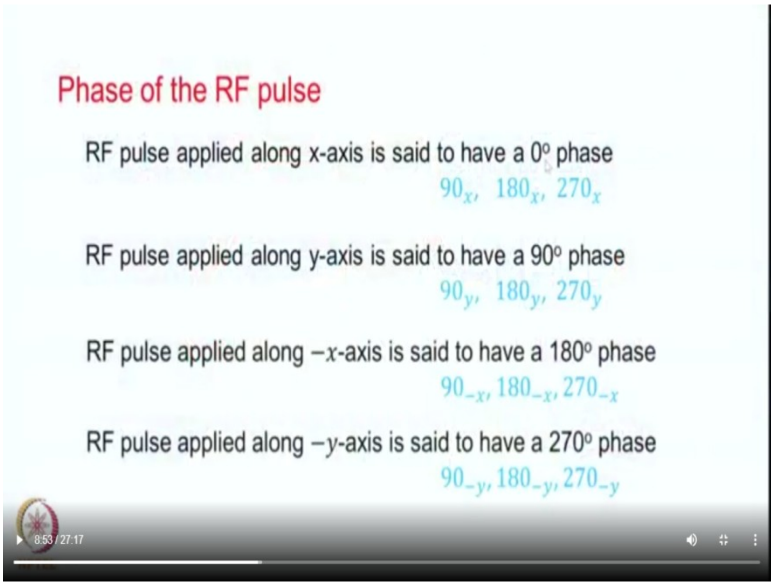

Phase of the RF pulse

RF pulse applied along x-axis is said to have a 0° phase

90_x, 180_x, 270_x

RF pulse applied along y-axis is said to have a 90° phase

90_y, 180_y, 270_y

RF pulse applied along $-x$-axis is said to have a 180° phase

90_{-x}, 180_{-x}, 270_{-x}

RF pulse applied along $-y$-axis is said to have a 270° phase

90_{-y}, 180_{-y}, 270_{-y}

Okay, now the next concept here is the phase of the RF pulse, when you apply the RF pulse applied along the X-axis it is said to have zero phase. So we may apply a 90˚*x* pulse or a 180˚ *x* pulse or 270˚*x* pulse, what do these things do? 90˚*x* pulse rotates the magnetization into the minus Y-axis 180˚*x* pulse inverts magnetization from the Z-axis to the minus Z-axis and this 270˚*x* pulse rotates the Z magnetization into the Y-axis. So therefore the it becomes important to define where the along which axis the pulse is applied. Essentially, this is the phase, this is the phase of the *RF* and depending upon that we have we call the pulse as a 90˚*x* pulse or a 180˚*x* pulse or a 270˚*x* pulse.

We can also have somewhere in between you can have a phase of the pulse at 45˚in which case it will be somewhere in between X and the Y- axis and you can control that and all this is now possible because of the computer which will allow you to control the phase of the RF because I you remember RF is also an electromagnetic wave and therefore it has a certain amplitude and a phase and we can always control this by the computer. So technically we say if it is a 0 phase we say it is along the *X*-axis or 180˚pulse along the *X*-axis 270˚ along the *X*-axis and so on.

Likewise, if you apply a pulse along the Y-axis then it is said to have a 90 degree phase. So like therefore we can have a 90*y* pulse, 180*y* pulse or a 270˚*y* pulse. Now, if you do the same thing with 180˚ phase means we apply it along the -X-axis, then we write it as 90 -*x*, 180 -*x*, 270 -*x* and so on so forth. Often, you will see a different kinds of nomenclatures used in the literature or in books people may write it as 90x or y or minus x or minus y but sometimes they may also use it as 0, 90, 180, 270 and so on.

So all of these mean the same thing and this is the correlation between the phase of the *RF* pulse and on which axis the pulse is applied.

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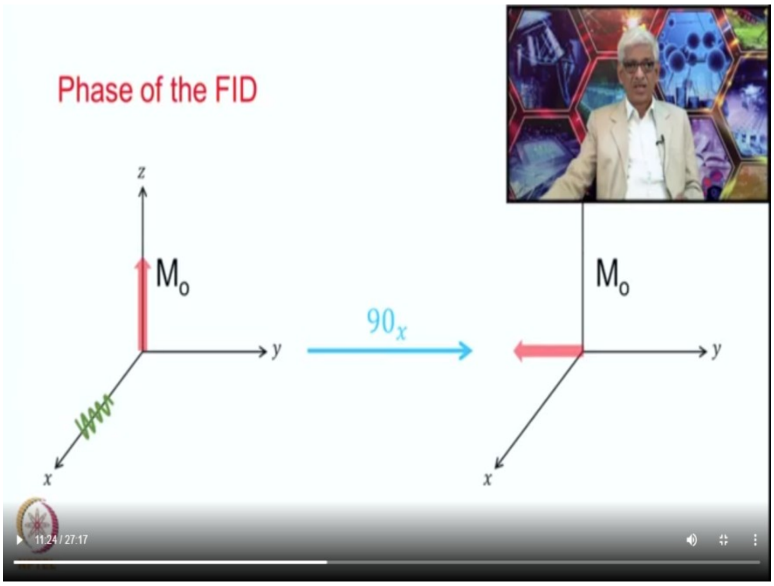

Phase of the FID

Okay, now what happens is a consequence of this. Let us begin looking at the generation of the FID once more, we have the initial magnetization along the *Z*-axis equilibrium magnetization. And now you apply a 90° pulse along the *X*-axis. So therefore as I said before the 90*x* Wells rotates the magnetization to the minus *Y*-axis, okay, and after that you collect the FID, you collect that FID.

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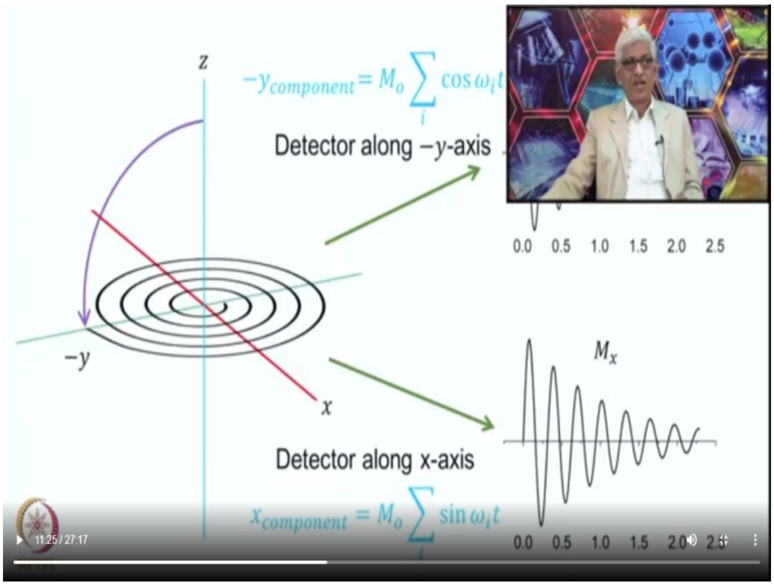

$-y_{component} = M_o \sum_i \cos\omega_i t$

Detector along −y-axis

Detector along x-axis

$x_{component} = M_0 \sum_i \sin\omega_i t$

What do you get? Now the magnetization is recovering back to equilibrium by rotating in this manner, it is a spiral way, it goes in the spiral way and eventually it will reach to the *Z*-axis. So while it is doing so you are collecting the *x* and the *y* components in your receivers. So the detector along the *Y*-axis, let us say how will the FID look like. So this magnetization is maximum here.

Okay, when it is here, the *x* component of the magnetization is 0 so if I look at the *y* component of the magnetization as it is moving and recovering it follows this sort of a pattern, there is also decay there is a transverse relaxation decay happening and therefore the FID decays like this. In this of course the decay is not written but the there will be an exponential factor which causes the decay here, this is the frequency component which is written here. So, exponential decay will happen at this point and therefore you will have the signal going like this.

So we may want to add that here to indicate the decay as well. So here if you multiply this by e^{-t/T_2} and here also we multiply e to the -*t* by T_2. This is the transverse relaxation here and this allows for the decay of the signal. So when you do that to the magnetization collected along the X-axis starts, so in this case the magnetization starts from here and it goes down to 0 and then oscillates in this manner.

But look at the x component of the magnetization and this starts from 0 because initial point x component is 0 and there as it moves around here, then this picks up and then it goes in the sine wave here as in this manner and also it will be decaying because of the exponential $\frac{-t}{T_2}$ factor.

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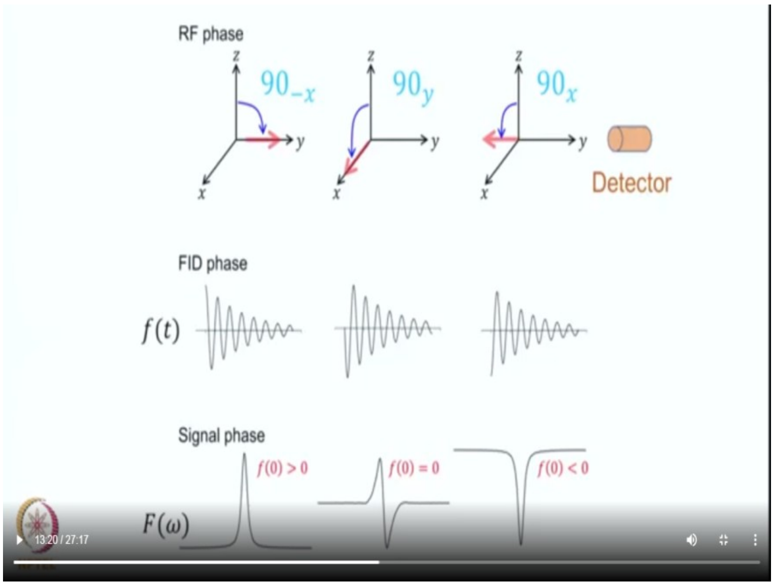

We can apply various kinds of pulses along with the different phases and let us look at what it results in terms of the spectrum. If I apply 90 -x it comes along to the Y-axis, if I apply a y pulse it comes along to the X-axis, if I apply 90x pulse which goes to the minus Y-axis as we indicated earlier. Now what will be the nature of the FID in these cases? I am detecting the y component, so this FID goes in this manner in the same way as I indicated before but here the signal goes in this manner as I indicated before and here what happens this is the x pulse this goes from -y and it goes in this manner.

So this is exactly 180° out of phase with respect to this. So this is the way it goes in this manner. Now if I were to fully transform this signal so I get an absorption signal in this manner which has maximum intensity you remember in one of the Fourier transform theorems the first data points essentially is the integral of the spectrum and here the first data point is the maximum it is positive this gives you the total integral of the spectrum. This is the integral of the spectrum which is positive which is greater than 0.

In this case when the signal is collected in this manner, it starts from 0 here $f(0)=0$ and this produces the dispersive signal and the integral of dispersive signal is 0. This half and this half are opposite in sign and therefore this goes to 0 and here when it starts from the negative side, obviously, it will have a negative integral and this goes in this manner and $f(0)<0$. So therefore we call this as signal phase, if I call this as 0 phase this is 90°phase and this is 180° phase. So this is the relation between the *RF* phase and the signal phase and the FID phase, all these things are correlated in some manner.

Okay, now with regard to the detection, what kind of frequencies we get when we actually collect the data and Fourier Transform. So suppose we collected the cosine component only we call that as the single channel detection. So now if it is a cosine component which is getting detected, so then my FID is cosine omega t, right?

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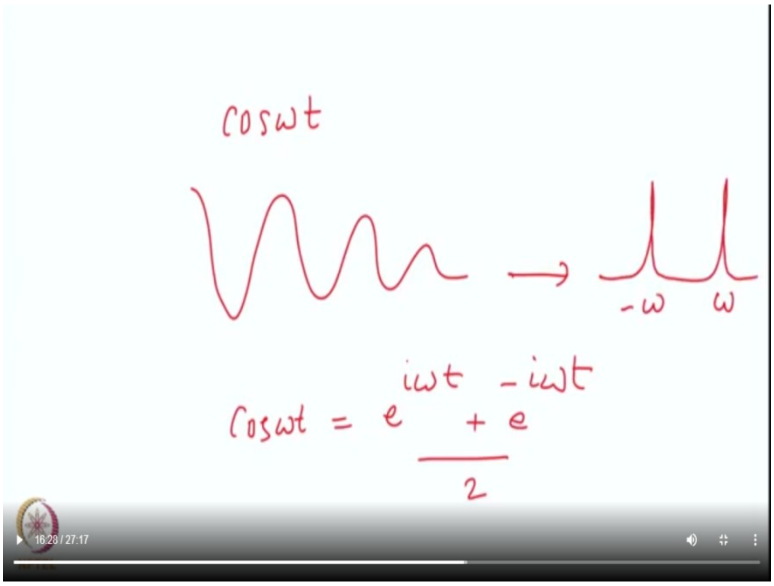

And this FID is going like this as we saw, now when a Fourier transform this what do I get? Now if you remember $\cos\omega t$ can be written as

$$\cos\omega t = \frac{e^{i\omega t} - e^{-i\omega t}}{2}$$

Therefore, there are two frequencies here plus omega and minus omega and therefore when a Fourier transform this I will get two signals. And if this is $+\omega$, this will be $-\omega$ and that is what is indicated this slide here.

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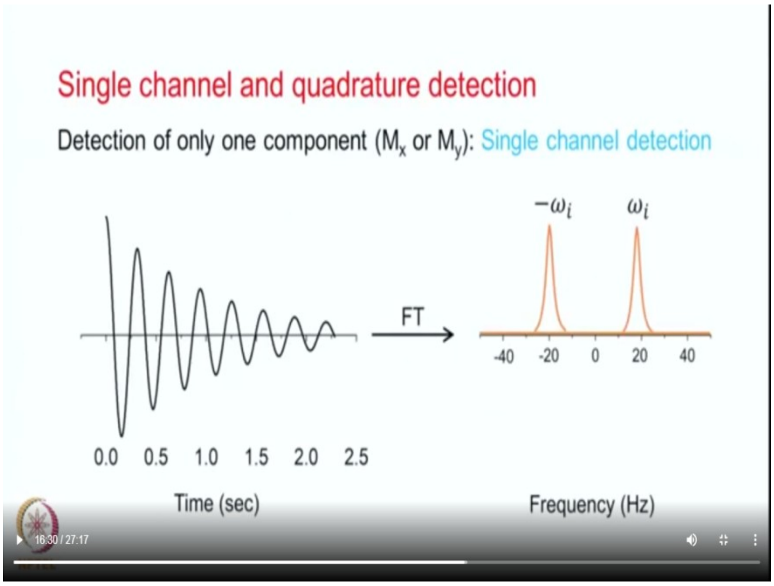

Single channel and quadrature detection

Detection of only one component (M_x or M_y): Single channel detection

So I have here when a Fourier transformation if it is a cosine function it is absorptive to signal and I will have two frequencies $\omega_i, -\omega_i$ and this is called as a single channel direction. If I were to detect x component of the magnetization, this is for the y component if I have to detect x component, this will have also a $+\omega, -\omega$, but they will have dispersive phases like this one like this and the other one like that. So they will have also plus and minus frequencies in the same phase.

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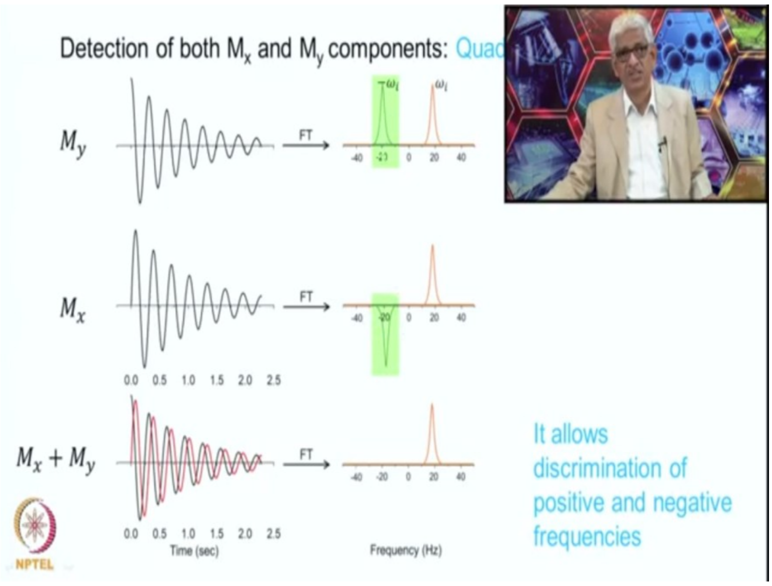

Detection of both M_x and M_y components: Quad

It allows discrimination of positive and negative frequencies

Now, this is indicated here, now, if I have to detect both of the components M_x and the M_y components and this is called as quadrature detection, okay. Now quadrature detection y component is giving me these two frequencies as $+\omega, -\omega$, M_x component is giving me again two frequencies but notice however that I have written here is absorptive signals, but this comes as a result of phase correction. After I do a certain 90° phase correction on the dispersive signals which we collect with the M_x component then I get this absorptive phase because there is the between the absorptive face in the dispersive line shape, there is only 90 degrees phase shift, so I add this 90°phase shift then I will get this negative here and this positive here.

Then what I do is I add these two frequencies, this is $M_x + M_y$ both are collected these that collected separately in Fourier transformation and then added then you get you have cancellation happening of these two components, whereas this one survives and therefore I will have only one signal present here the and another signal is removed because this signal was an artifact of Fourier transformation, those are not really present in your spectrum, it was an artifact of Fourier transformation, we do not want that.

So therefore we have to remove it and we have, for that we have to do this separately, we will have to collect both the components separately Fourier transform them and do a phase correction on one of those components and when you add it this removes this component and you will have a proper signal at the desired place. This is called as quadrature detection.

Now and this for this allows discrimination of positive and negative frequencies. So if I have the offset carrier frequency at one place, then this will allow me to discriminate whether I have my signal I on the right side of it or the left side of it and because the right side and the left side they will be called as positive and negative frequencies and they will be discriminated when we have a quadrature interaction. If I want to call this as positive frequency, this is the negative frequency and if these are real present in your sample, then you will be able to discriminate them using quadrature detection.

But sometimes some artifacts appear, what are these artifacts? So let me also write that here artifacts are called as quadrature images.

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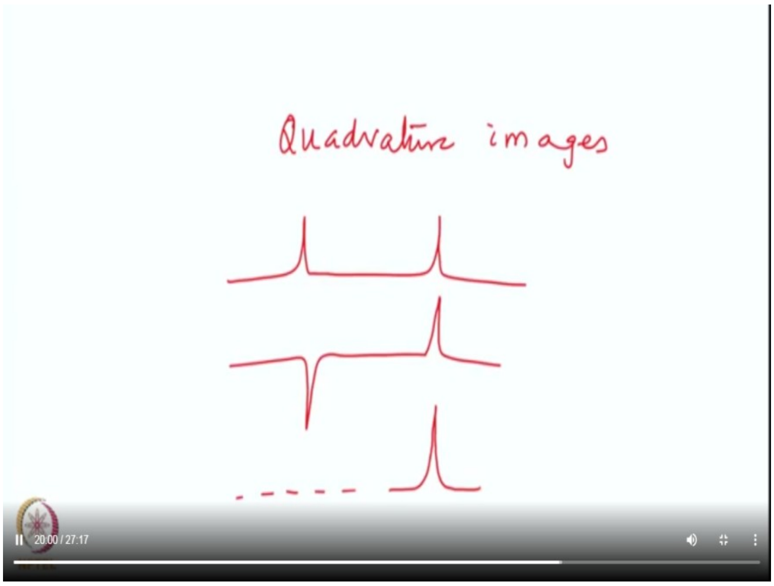

So we said that this a signal and I will have a signal like this, the axis when I add this this will go and I will have only signal like this, but this assumes that these two signals are exactly identical because these are collected with the two different receivers, one is along the *Y*-axis other one is along the *X*-axis. Suppose there is a mismatch between these two receivers and the signal amplitude here is not the same as the signal amplitude here, then it will produce a small glitch here and some and which does not have any particular phase or anything like that and this is called as quadrature image.

And this has to be removed and this up there are of course techniques to remove this kind of glitches. And this quadrature images are not desirable in your spectrum. So that is a special feature of Fourier Transform NMR and then we have the next thing that comes out is what is called a signal digitization and sampling. We set the signal has to be sample and at regular intervals of time.

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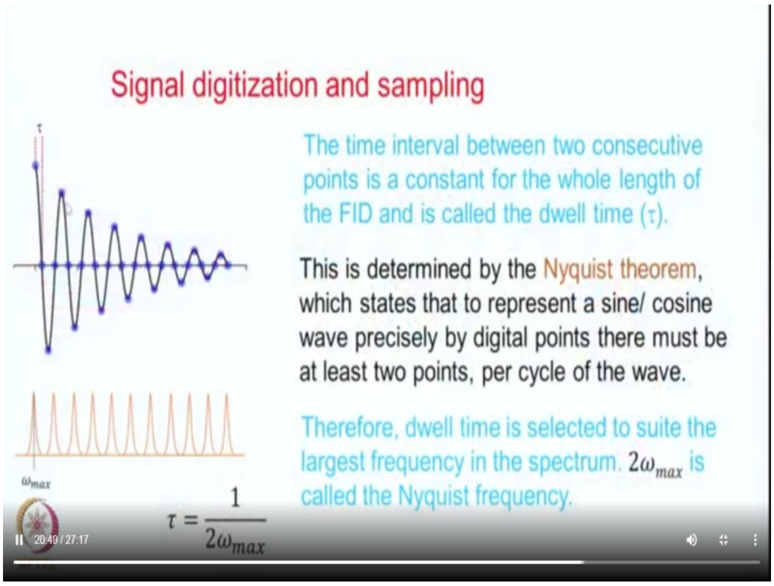

Signal digitization and sampling

The time interval between two consecutive points is a constant for the whole length of the FID and is called the dwell time (τ).

This is determined by the Nyquist theorem, which states that to represent a sine/ cosine wave precisely by digital points there must be at least two points, per cycle of the wave.

Therefore, dwell time is selected to suite the largest frequency in the spectrum. $2\omega_{max}$ is called the Nyquist frequency.

$$\tau = \frac{1}{2\omega_{max}}$$

So this is a few collected data point here, a point here, point here and so on so forth at regular intervals the time between two successive points is called as the dwell time, okay and suppose I want to represent it as tau but do not confuse this with the pulse width which also we said earlier as τ, but you can well you can call pulse width as τ_p if one likes but this is now at the moment this is the dwell time what we are talking about. So this is the time between two consecutive points is the same between every two points.

Now how do we decide what should be this dwell time? Okay. Now this is determined by what is called as the Nyquist theorem. Now, what is the Nyquist theorem? This states that to represent a sine or a cosine wave precisely by digital points there must be at least two points per cycle of the wave. So if I have a full cycle of the wave, if I have a wave going like this in that way there must be at least two points. So that is properly represented and that is called as the Nyquist theorem, and therefore what frequency we should therefore sample?

Therefore, the dwell time is selected to suit the largest frequency in the spectrum. If omega max is the largest frequency in my spectrum then I must collect at the rate of $2\,\omega_{max}$ because two points per cycle means $2\,\omega_{max}$ should be the frequency, that should be the ray. So that many points I must collect per second. That is the rate and this is called as the Nyquist frequency.

Therefore, ¿$\frac{1}{2}\omega_{max}$, okay? So if I choose this naturally every other frequency, which is smaller than this will have more than two points therefore every frequency will obviously be represented in this kind of a choice. So you choose your frequency to suit to the largest frequency in your spectrum. If I collect these many points, then naturally I will be collecting more points for each of the frequencies. Well to explicitly show it let me indicate to you how.

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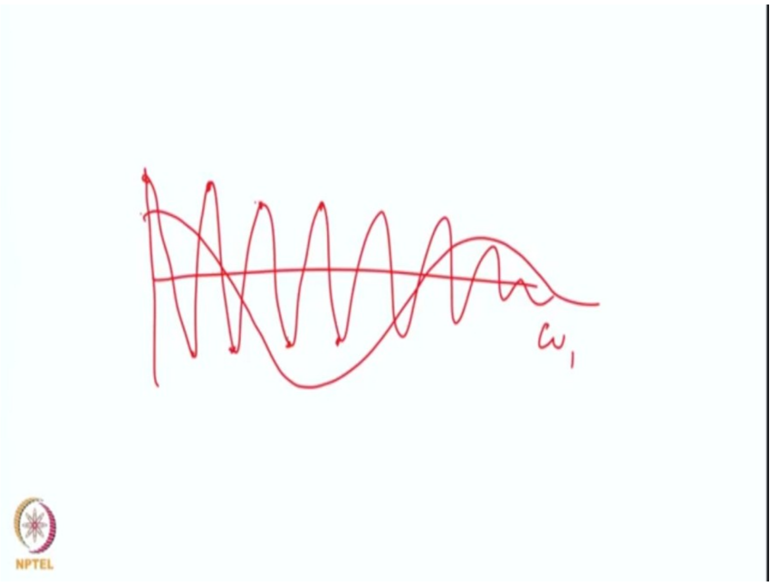

So if I have a FID which is going with the frequency like this, okay? And this is the last is the particular frequency called as let us say omega 1 and I have points here at least two points per cycle 1, 2, 3 so this is the largest frequency let me say I am collecting two points per cycle. Then I have another frequency which goes let us say like this, okay?

So now how many points I have per cycle? From here to here I will have 1, 2, 3, 4, 5, 6, so 7 up to so many points I have. So any smaller frequency I will have I will have a large number of points represented, therefore since the data is collected as a result of the sum of all of these frequencies, so each point therefore represents all the frequencies correctly. Therefore choose the largest frequency for to define your sampling rate. That is my sampling rate and accordingly I will choose the spectral widths.

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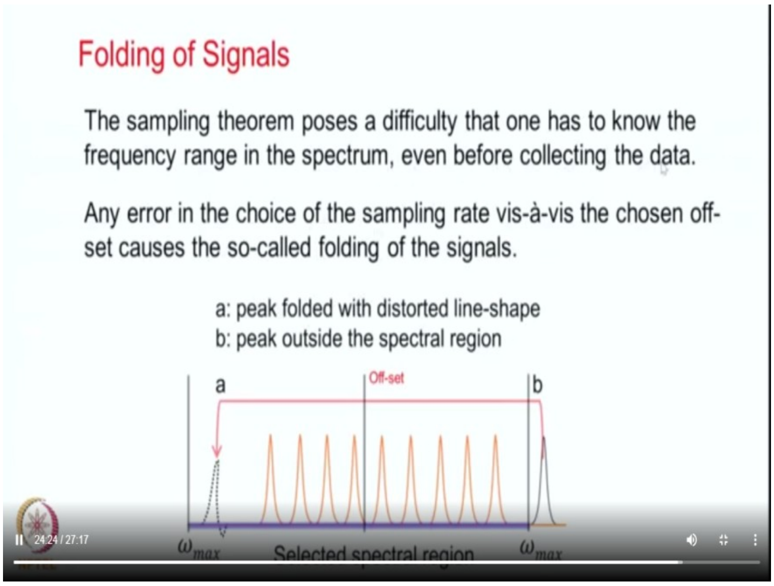

Folding of Signals

The sampling theorem poses a difficulty that one has to know the frequency range in the spectrum, even before collecting the data.

Any error in the choice of the sampling rate vis-à-vis the chosen off-set causes the so-called folding of the signals.

a: peak folded with distorted line-shape
b: peak outside the spectral region

So now another important feature that happens here is what is called as the folding of signals. The sampling theorem poses a difficulty that one has to know the frequency range in the spectrum even before collecting the data because you prior you do not know where your frequencies are.

Then how to place the frequency how to place your carrier, where to place the carrier. So, how to choose the offset, that is often a difficult problem. So therefore you can always end up choosing a wrong offset or a wrong filter and then you may not be able to cover your proper spectral range. In such a situation, what happens is the signals which are outside your spectral region get folded into your region of interest they appear with a different phase and this is a phenomenon known as folding of signal.

The sampling theorem poses the difficulty that one has to know the frequency range in the spectrum even before collecting the data and this is not an easy thing to do and we can get into troubles if the choice is not proper, okay?

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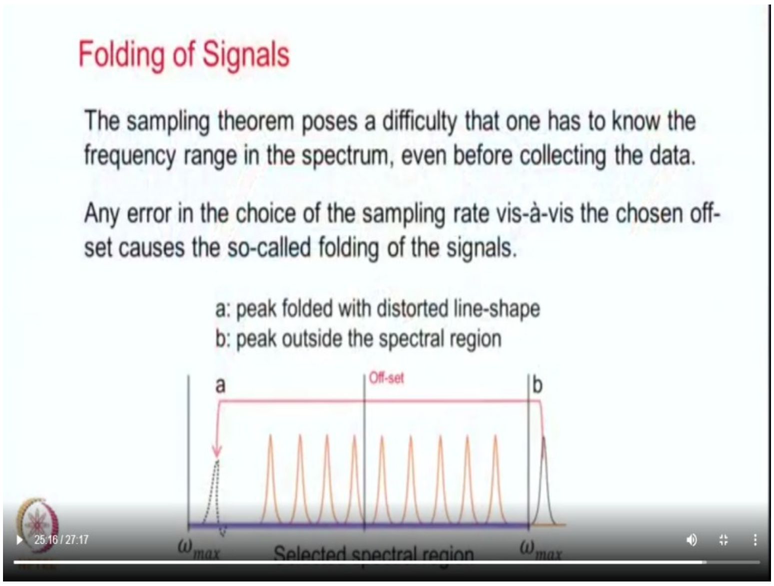

Folding of Signals

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Any error in the choice of the sampling rate vis-à-vis the chosen off-set causes the so-called folding of the signals.

a: peak folded with distorted line-shape
b: peak outside the spectral region

Any error in the choice of the sampling rate vis-a-vis the chosen offset causes the so called folding of the signals and this is illustrated here. Typically, that suppose I have a spectral region which I have selected here what I means I selected here then I applied my filter so that I select only this much is a region of my spectrum compared to the offset. The offset is here half of the spectral region is here, half of the spectral region is here. Therefore the largest frequency which I have is omega max and this accordingly I will choose my the dwell time, right?

So now if this is not proper that some frequencies are left out, for example this one if it is left out then it turns out that this fellow will fold into this region at this point and this is called as the folded signal and this appears as a result of some digitization problems, which we will discuss in the next class in greater detail. This is the consequence of folding, this is the consequence of wrong choice of the dwell time and wrong choice of the spectral width or the filters which we are giving for the data collection, okay?

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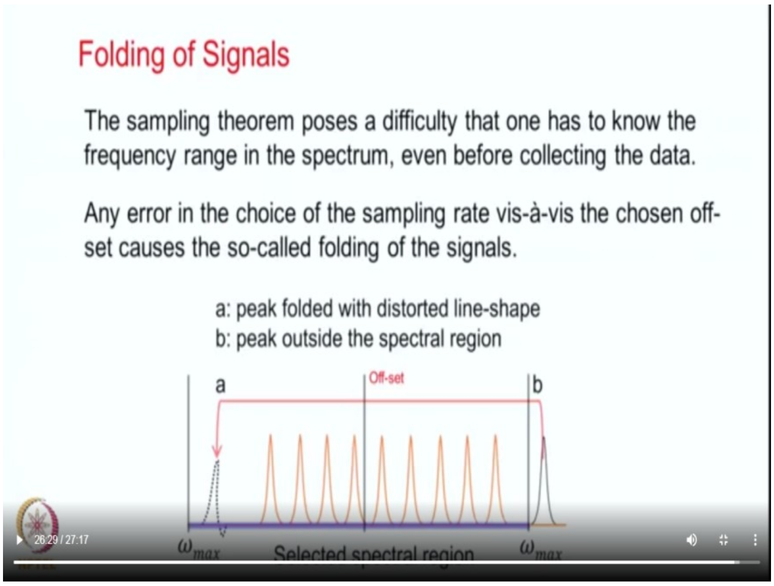

Folding of Signals

The sampling theorem poses a difficulty that one has to know the frequency range in the spectrum, even before collecting the data.

Any error in the choice of the sampling rate vis-à-vis the chosen off-set causes the so-called folding of the signals.

a: peak folded with distorted line-shape
b: peak outside the spectral region

So this one digitization is indicated here and folding of the signals happens as a result of this and we will continue with the folding of the signals in the next class, thank you.