NMR spectroscopy for Structural Biology NS
Prof. Ashutosh Kumar and Prof. Ramkrishna Hosur
Department of Chemistry
Indian Institute of Technology - Bombay

Lecture: 09
Practical Aspects of FT-NMR-I

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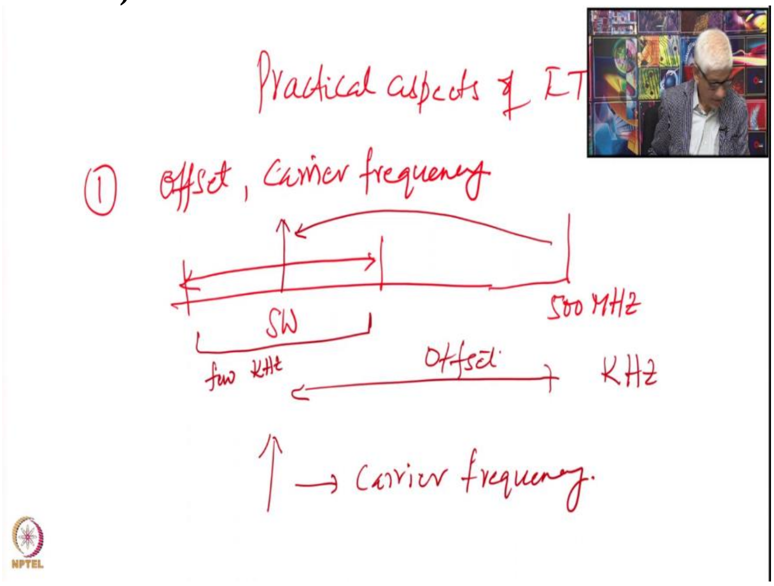

So, let us now look at the practical aspects of FT-NMR. First offset or it is also called as the carrier frequency. So, what is the meaning of this? Now we are exciting all the frequencies in one go here right. So, let us say I have a spectral region from here to here this is my spectral region and my spectrometer frequency is somewhere here let us say this is 500 megahertz. If I monitoring the protons actual region of the spectra is here which is little away from here somewhat away we do not know how much away.

Because we do not know how much after you apply the current where the magnetic field has come and what is the exact frequency of the spectrometer some arbitrariness will be there in that one. So, therefore nonetheless what we have to do is we want to excite the region of our interest. Region of our interest means you remember with respect to the spectrometer frequencies we are choosing a few kilohertz into the left hand right of that one.

Therefore we would like to have that spectrometer frequency here we would like to have that spectrometer frequency here right. So, what we have to do is we have to shift this to this place in other words we have to add a certain amount of offset to this frequency and that the amount of shifting what we do this is called as the offset. So, from here to here what we are shifting this is called as the offset.

This is in kilohertz some kilohertz it will be it will not be megahertz it will be some kilohertz. So, 5000 kilohertz I mean 5000 hertz or 6000 hertz and things like that that much of range will occur and this of course is few kilohertz. So, when we do this we achieve a uniform excitation of all your frequencies therefore it is necessary to move the spectrometer frequency to this point. And then this frequency what I put here this frequency this is called as the carrier frequency.

Spectrometer frequency is here main frequency but when I shift it then I bring it to the center of my region or one end of the region or whatever one end of the region if I bring it then it is called as the carrier frequency and amount of which I shift is called as the offset.

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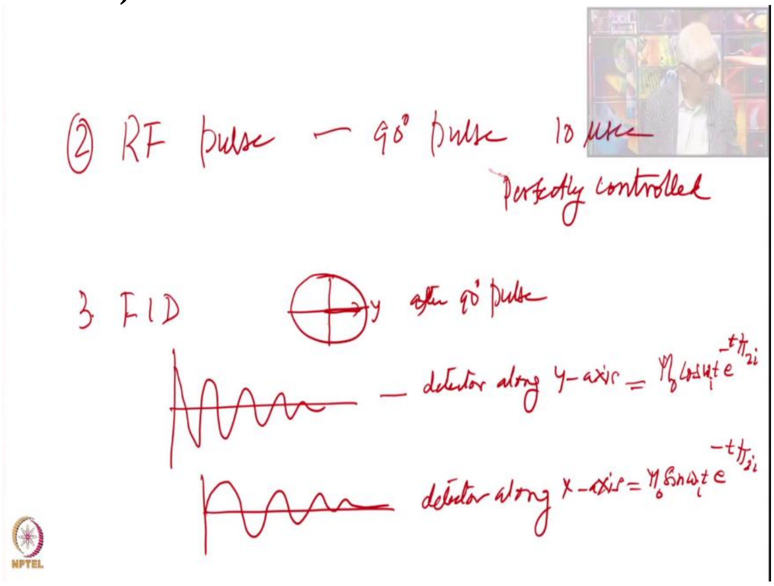

Then the next one is RF pulse; RF pulse we said we have to apply 90 degree pulse. So, this may be let us say 10 micro seconds. So, you must control it very well it has to be exactly 10 micro seconds not 11 not 10.5. So, therefore perfect control is required in this RF pulse, pulse width. So, 90 degree pulse means 90 degree pulse and that has to be perfectly controlled. And then so then I have the third part is the free induction decay.

Free injection decay which we already saw this; what how does it look like. So, we said there are 2 ways I said if I monitor the y component of the magnetization. So, magnetization has come here after the 90° pulse. This is let us say this is y this is after 90° pulse. Now depending upon where I put the detector if I put the detector along the Y axis or along the X axis.

Suppose I have one detector I put it along the y axis. So, then my FID will look like this. So, this is detected along Y axis. Now if I put the detector along the X axis. So, where it will start because at X axis at the time t = 0, x magnetization is 0. So, as it starts rotating here then it is start x magnet starts building therefore it is like a sine wave this one is like a cosine wave this is $M_0 \cos \omega_i t\, e^{-t/t_{2i}}$.

And this one will start from 0 and then it will go like this, this is detected along X axis and this will be this signal will be $M_0 \sin \omega_i t\, e^{-t/t_{2i}}$this is for the individual one particular frequency similarly for the other frequencies as well. So, what is the consequence of this?

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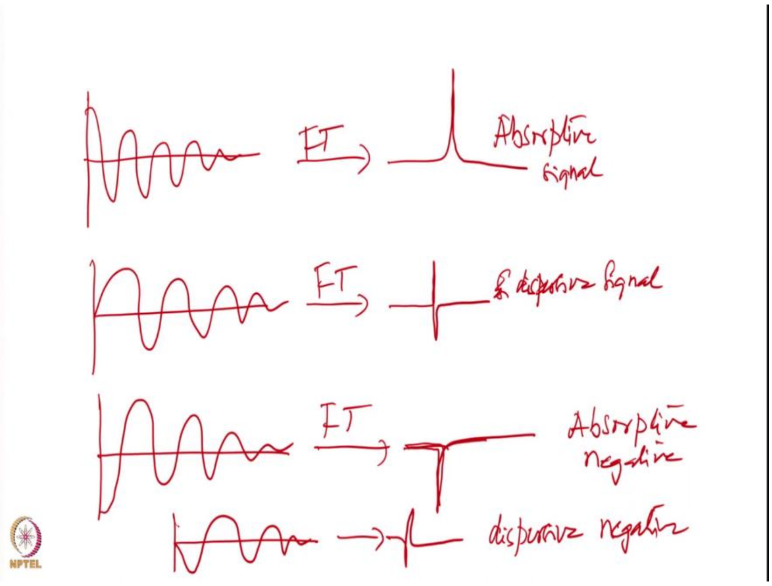

So, if I were to Fourier transform this if a Fourier transform in FID like this, this FT will produce me a signal which is like this and if a Fourier transform an FID which is like this it will produce me a signal like this. So, this is my absorptive signal and this is a dispersive signal. So, correspondingly you can also have the negative signs of these ones also in other words if my if I did to show you one if I collect an FID which is like this starts from the minus value then I will get a signal which has a negative sign.

So, this is absorptive negative and what is dispersive negative. So, let me also draw that one here dispersive negative will be it will go like this. So, this will produce if I draw like that and this will be this is dispersive negative. So, typically depending upon how we actually collect your signal you will have different kinds of line shapes in your spectrum these are the important consequences as we will see in the practical aspects later.

So, this is with regard to the FID and then the signal. And now let us look at the detection system.

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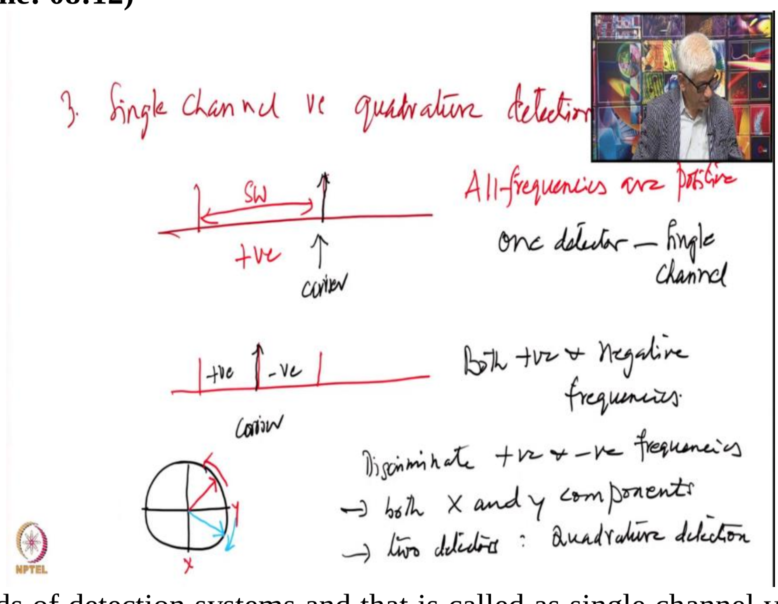

There are 2 kinds of detection systems and that is called as single channel versus quadrature detection. So, now first let me see the single channel. So, this is my spectral range I bring my carrier here, this is my carrier this is the spectral range here this whole region is mine this is a spectral range. So, therefore with respect to the carrier all frequencies are let us say this is positive.

All frequencies are positive. Suppose I do an experiment where this is again my same spectral width here if I bring my carrier here what that means here I have positive frequencies here and negative frequencies here. So, we have both positive and negative frequencies. So, when you have this sort of a situation all frequencies are positive you need to have one detector. So, one detector because all frequencies have the same sign.

So, there will be no difficulty there will only one kinds of signals which are present there. Now this is called as single channel because it is one detector this is called as single channel. Now here if I have to discriminate between positive and negative frequencies because it is necessary to discriminate between positive and negative frequencies right. So, how do we do that?

So, if you are to do this I will have to discriminate positive and negative frequencies I need to collect both x and y components how is why is it. So, so let me draw that here what is the meaning of positive and negative components. So, positive components meaning they go in this direction and negative components mean they come in this direction right. So, therefore so, this one this will go like this, this will go like this. So, these are positive negative frequencies.

So, if I want to discriminate these 2, I have to collect both the y component and the x component let me draw here the x and y. So, if I collect both of the x and the y components then I know exactly whether it is a positive frequency or negative frequencies. At the same time I should collect both the components at the same time. So, if I have to do that I will need. So, this will need 2 detectors and therefore this is called as quadrature detection.

Both the detectors have to be identical and they will collect the signal at the same time the FID is as it is growing you collect both of the x and the y components simultaneously you process them and then you will be able to see the single frequency as it happens. So, that is the positive and negative components we will be able to distinguish between those 2.

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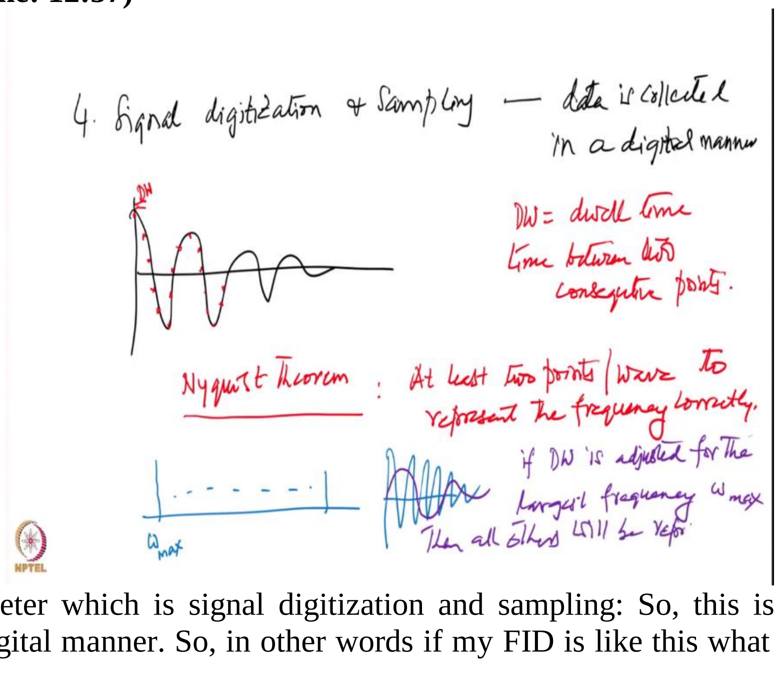

The next parameter which is signal digitization and sampling: So, this is because data is collected in a digital manner. So, in other words if my FID is like this what I will be doing I will be collecting data here, here, here, here, here and so on. So, digitally it is collected with time intervals in between okay. So, this is called as a signal digitization times between 2 consecutive points has to be the same and this time if I went to represent like this here let me call this as dwell time.

So, this is the time between 2 consecutive points. Now of course we ask this question how do, we know this; what should be the time between 2 points I have the FID, FID has various frequencies. So, what should be the time in between. So, this is where a particular theorem called as Nyquist theorem comes into the picture Nyquist theorem that is it says you need at least 2 points per wave to represent the frequency correctly this determines what is called as the sampling.

Sampling meaning how you collect the data individually and that is called as sampling. So, the Nyquist theorem tells me how many data points I should collect. So, if I have a larger number of frequencies in my spectrum. So, I have my carrier here let us say and I have this largest frequency here ω_{max}. This is the largest frequency I have various other frequencies in between here.

So, if I adjust my sampling. So, that I have at least 2 points for the ω_{max}. So, this largest frequency meaning what let us let me draw the adjust frequency here if this is my largest frequency. How will the frequency is smaller than that will go. So, let me draw that also here. So, a slower frequency will go like this right and even slower will be even slower than that. Therefore if I have at least 2 data points to represent the largest frequency automatically there will be more data points for the lowest.

If dw is adjusted for the largest frequency ω_{max} then all others will be represented. So, this is the Nyquist theorem. And therefore this is the signal digitization and the sampling. So, these are all very specific to FT-NMR as you can see. So, now a consequence of this will be suppose you make certain mistakes that you do not choose this dwell time properly and then you are not covering all the frequencies correctly. So, what will be the consequence of this? So, this leads to what is called as folding.

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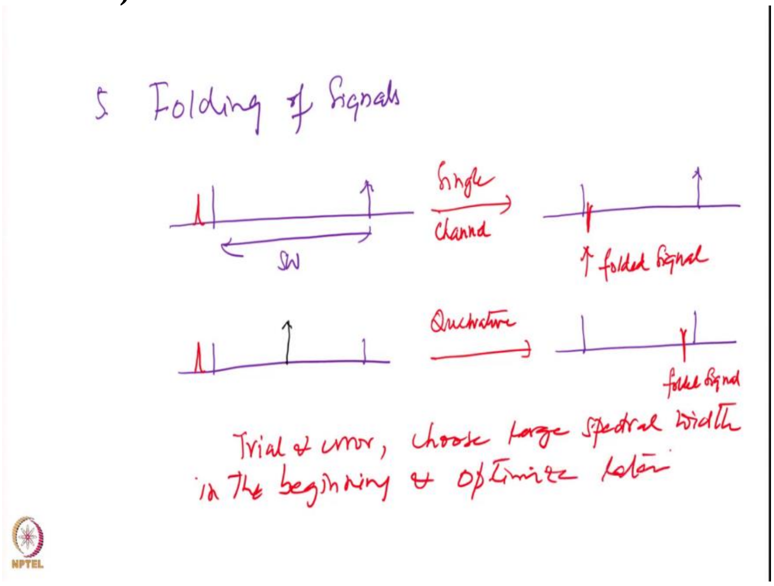

Folding of signals folding of signals and that is the following suppose I choose a carrier at this point and my spectral width up to here I choose a spectral weight because I choose a dwell time according to that. So, this is my spectral width this is what I assumed it has the largest circular number of frequencies. Because the priori you do not know you probably not know what is the range of your frequencies you arbitrarily choose some numbers, that my frequencies are supposed to be present within this area.

Suppose I have a signal you chose your dwell time according to this if you choose that then suppose I have a frequency which is present here you did not realize it. You did not realize it there is a frequency which is outside the region which you have chosen. So, what will be the consequence of this if you are doing single channel detection. Then in your spectrum this is this was the region which you chose right.

This was the region which you chose and this last fellow which you have missed it that will appear inside here with a invert some distorted phase it will appear with it distorted phase meaning which will be neither purely absorptive or not purely disperse you it will have some sort of a distortion there. So, this is a folded signal this is called as folding. On the other hand if you are doing a quadrature detection. Now where is my carrier the spectral range is this much right.

Let me draw the spectral range same as here but now my carrier is in the middle my carrier is in the middle that I can detect both positive and negative frequencies right I can discriminate between positive and negative frequencies and in this situation if I have I made a mistake in choosing the offset and I have a signal which is present here. Then where will this appear this will also fold this is quadrature.

Then it will appear here this was present at this point. So, which was outside here in this area and now it will appear on the other side here. So, this is the effect of quadrature detection and this is the folded signal and therefore this is an important factor one has to take care. How do we know this that when you detect a folded signal? So, what one should do. So, what one should do is to by trial and error choose a large spectral width in the beginning and optimize later the next point.

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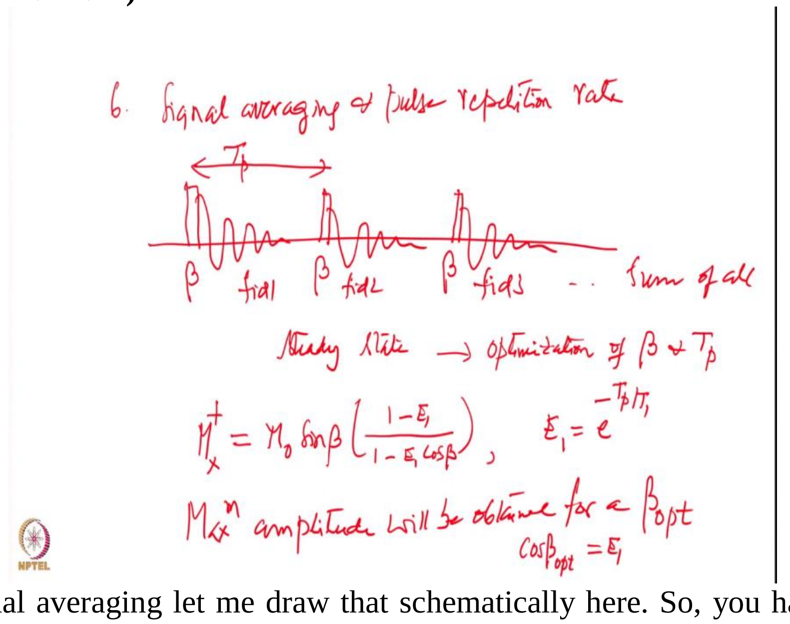

So, what is signal averaging let me draw that schematically here. So, you have the first RF pulse you collected FID wait for the time certain time again RF pulse collect FID wait for a time third RF pulse collect FID. So, this is FID1 FID2 FID3 and you are going to take the sum,

sum of all let us say the time between these two is T_p time between 2 pulses is the same everywhere. So, this has to be the time.

What should be the optimum time and let us say this is the; I have the flip angle beta here this is also beta this is not necessarily always be 90° I have to keep the same. So, when I apply in the pulse what happens the I collect the FID all right that is the decay of the transverse magnetization. But the longitudinal magnetization may not have recovered completely because this is dependent on the T_1.

T_1 the spin lattice is relaxation time. The longitudinal magnetization may not have recovered completely. So, therefore when I apply the next pulse the starting point for the next pulse is not the same as the starting point for the first pulse. Similarly for the third pulse it may not be the same as the second and so on and forth. But we would like it to have the same every time it should be the same. Therefore what we should do we should have a steady state at steady state it should be the same for every one of those.

After that you start collecting the signal I mean you collect the signal nevertheless. So, you have to reach a steady state as early as possible. So, that every time it is that the same. So, therefore this requires optimization of the flip angle beta and Tp. So, let me write here the expression for that your x component of the magnetization after the pulse at the after the steady state is reached and the steady state is reached is equal to $M_x^+ = M_0 \sin\beta \left(\frac{1-E_1}{1-E_1\,cos\beta}\right)$ what is E_1?

$E_1 = e^{-T_p/T_1}$. So, you get maximum amplitude will be obtained for a flip angle which is lit is called as beta optimum for a beta optimum. And what is $\beta_{optimum}$ we can see this, this will be given by which is given by cos $\beta_{optimum} = E_1$ and E_1 is dependent on the T_p and the T_1. So, if you optimize your β such that $\cos\beta_{optimum} = E_1$ then you get the maximum M_x^+. So, maximum x plus means you get the maximum signal to noise.

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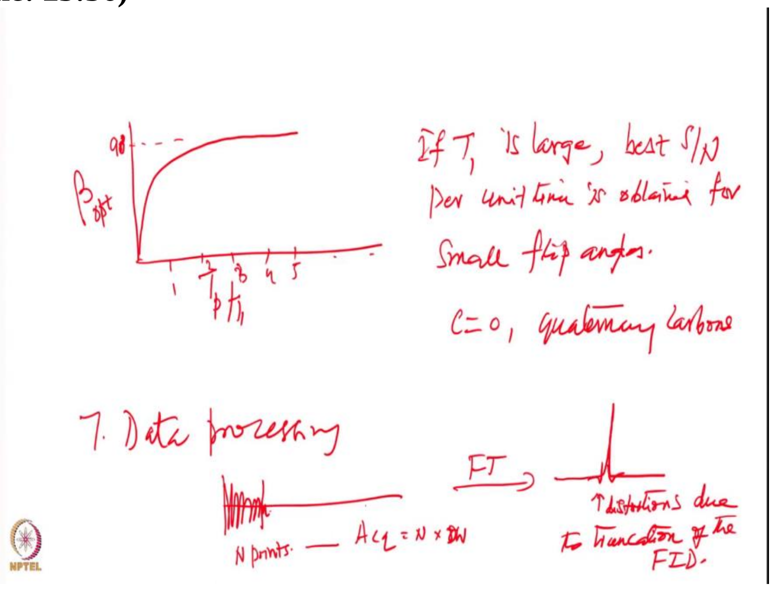

$\beta_{optimum}$ for $\frac{T_p}{T_1}$ So, notice that when you plot this curve it is for every one every point on this curve you have the maximum signal every point here you will get the maximum signal. So, therefore if my T_1 is very large compared to T_p what I should do. Now this one is 90° this is a

90°. So, all other ones are lower than that. So, so if the T_p is 5 * T_1 let us say this is 1 2 3 4 5 and so on so forth 1 2 3 4 5 etc. $\frac{T_p}{T_1}$ if T_1 is very large then I will have to use a very large T_p.

Suppose T_1 is 5 seconds then I will have to use a T_p of 25 seconds which is a very large time. Therefore the advantage of Fourier transform NMR will be lost when if you are doing that. But if T_1 is very short then of course you can use if T_1 is 0.1 second that can easily use 0.5 seconds for the T_p therefore there is no difficulty for that. Therefore if T_1 is large then best signal to noise per unit time is obtained for small flip angles this is typically so for carbon 13s.

So, carbon 13 relaxation times are very long it is for example has a very long relaxation time because it has no proton attached to it and therefore carbonyl relaxation times are very long. Similarly whenever there is no proton attached then you will have or quaternary carbons they have a very long relaxation times. So, these are for example these are carbonyls or quaternary carbons they are very long relaxation times.

So, in such a situation what one should do we use a very small flip angle this is one more point we will have to consider here and that is the digital filtration data processing aspects. So, next is seven data processing data processing is the Fourier transformation basically but typically when you do the Fourier transformation you what we observe suppose I have an FID which is going like this and I do not wait for the entire the FID to decay.

I do not wait until it goes down to 0 suppose I truncate the FID here I stop here because I priorly that I do not know what are the T_2 values therefore I do not know where to stop typically I collect let us say 1024 points or 2048 points and things like that. So, depending upon the number of data points what I have here N points and the acquisition time will be acquisition time will be will be equal to N times τ, τ is my dwell time.

Let me write it as dwell time and times τ. Now if I do a Fourier transformation of this of such an FID there will be distortions at the baseline. Suppose normal signal has to be like this then I will get distortions here and here. So, these are distortions, distortions due to truncation due to truncation of the FID. So, therefore what we do here to eliminate this problems. So, we do what is called as digital filtering digital filtering.

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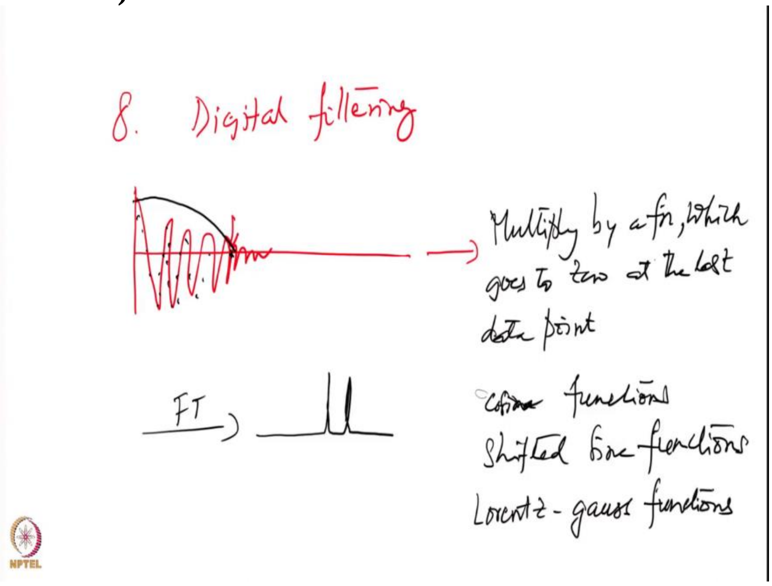

So, what is the meaning of this? So, I will do the same thing again here. So, then I have an FID which is going like that. Suppose I truncated the FID at this point. So, what I will do is I will multiply this by a function let us say which goes like this and brings it down to 0 exactly at that point multiply by a function which goes to 0 at the last data point. So, this results in elimination of those wiggles in your in the spectrum.

So, and then you will get a clean absorption line shapes when we do this then if you do after that if you do a truncation do FT then you get clean signals like this with no truncations here. So, this is digital filtering there are many functions which one can use you can use exponential functions. The functions can be cosine functions or shifted sine functions we will not discuss all of these things.

But depending upon the optimization one can choose different kinds of functions depending upon the number of data points you have. So, how many data points you will have here accordingly one has to choose because of course you have the data points here like that. So, the last data point is here you collected data point. So, it has to be 0 at the last data point you can use cosine function there is also what is called as Lorentz Gauss functions.

So, this we need not discuss in greater detail but this can be these are routinely available on the spectrometers. So, one can optimize which function to use and how much to use.

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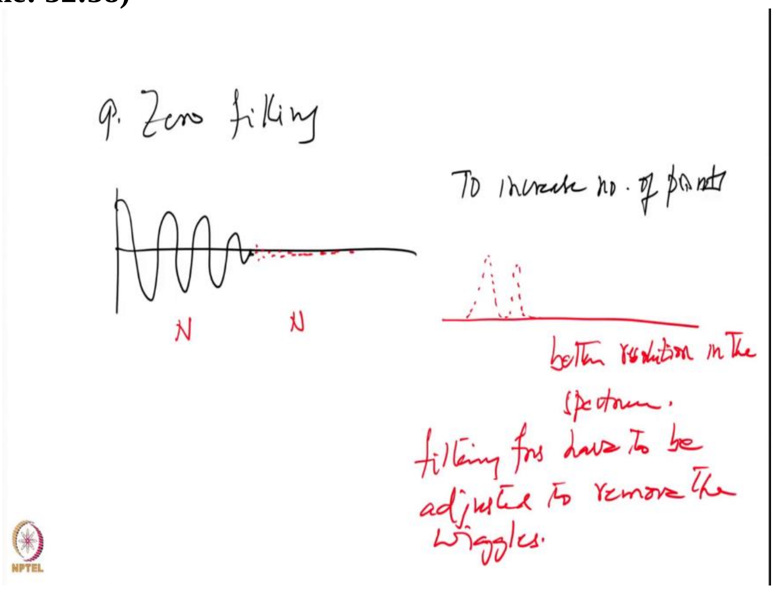

Then of course there is one another last one which I will mention that is what is called as 0 filling this is so your FID you are collected up till this point. Now what you do is to increase the number of points number of points in your spectrum. So, that the each signal is represented properly you fill in 0s I collected n more I add N more points here. So, therefore total number of points becomes to 2N therefore in your spectrum which is also in a digital form your spectrum which will be looking like this right.

So, you get a better resolution in the spectrum and of course when we do this the filtering functions have to be adjusted to remove the wiggles. So, I think we will stop here and continue with the next the next class.