NMR Spectroscopy for Chemist and Biologist
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Lecture 16
Data Processing in Fourier Transform NMR

Let us continue our discussion with Fourier transform NMR and its various aspects, we were talking about the various practical aspects of Fourier transform NMR and we have reached up to some aspects and one of the important aspect which we had started discussing was so-called folding of signals. You recall that the FID has points because we have collected the data in the digital form and the sampling theorem dictates how fast we should collect the data how the things should be digitalized and what should be the time in between the two consecutive of points and that is called as the dwell time and this is determined by what is our spectral width where we have kept our offset.

So, assembling theorem tells us the next frequency tell us that the sampling rate should be equals to $2\omega_{max}$, where ω_{max} is the maximum frequency in your spectrum, I mean ω_{max}not a W max, ω_{max} is the maximum frequency in your spectrum.

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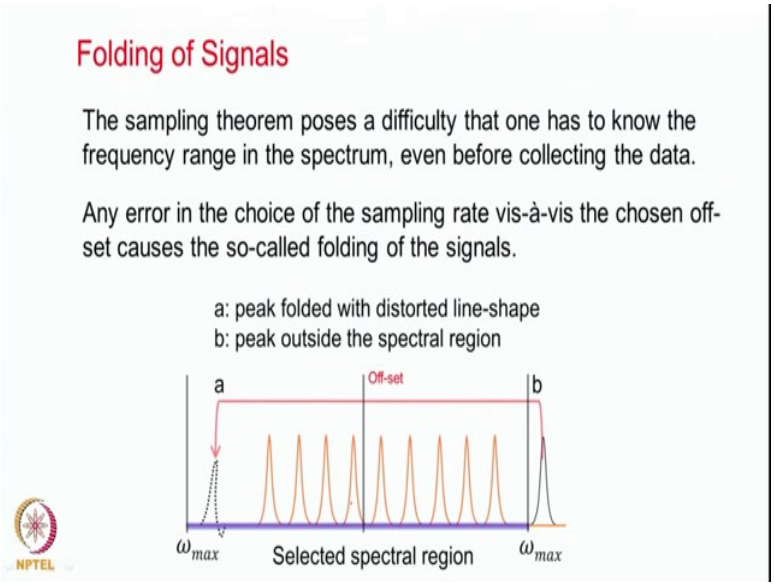

So, the sampling theorem therefore possess its difficulty that one has to know the frequency arrange in the spectrum, even before you collecting the data.

So, where to place the offset, what is the spectral range and what is the maximum frequency in that spectrum with respect to the offset is something one has to know, however this is not

easy to know, what happens as a consequences of this is that some artifacts appear in your spectrum, let me try and explain that little bit here and this is shown in this particular slide in a picturized way. So if you have a spectral width chosen from here to here, we can see that in my arrow that is from here to the blue this line indicates what is the spectral width you had chosen and if your frequency is kept offset is kept here and if there is a line somewhere outside here then it will fold into a spectral region into this and this is in the case of quadrature detection.

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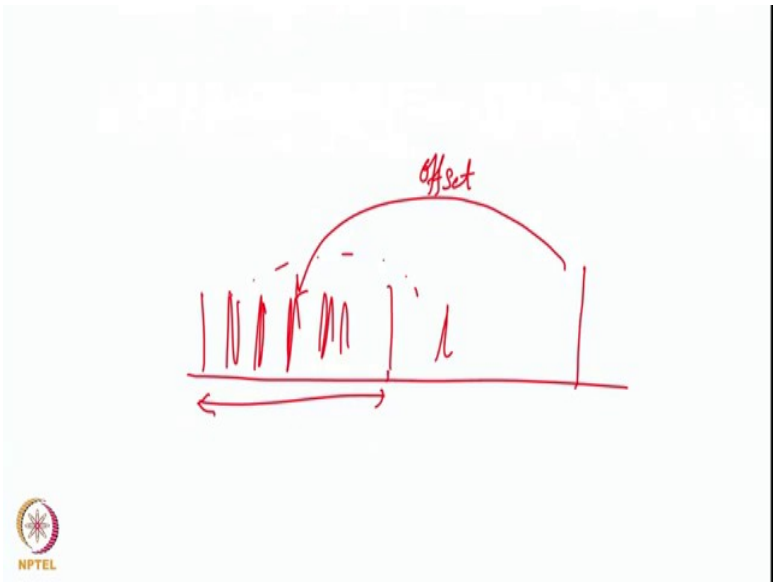

And the same thing let me try and explain, so if you have a this is your carrier frequency and your spectral region is here you have lots of lines here, here and here and here and here you think this is your spectral region, so therefore to excite this uniformly what you do is you shift your carrier frequency to the center of the spectrum here, this is what we set is called the offset, okay?

Now if this is properly chosen, then of course all of your signals will be inside the region what you have chosen this is the spectral region what you have chosen from here to here if the offset is properly chosen and you have chosen the spectral width which is determine by the sampling rate your dwell time what you have given then there is no issue but suppose you have a signal which is somewhere here which is not covered in this region which you have chosen. In that case this fellow will actually fold into your region of interest in some manner and that is called as the folding of the signal.

How does that happen? This happens as a consequence of digitization theorem which we discussed earlier and we will look at that once more. Of course the noise also we will fold

from here to here, it has its another consequence which will also discuss in the next few minutes, okay?

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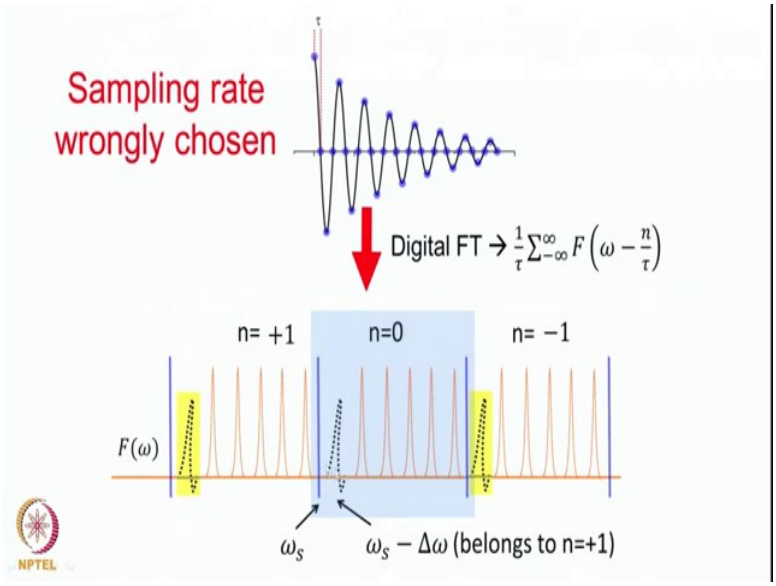

So what is the consequence of the digitization theorem? Sampling rate is wrongly chosen, as I mentioned to you earlier, we placed the offset somewhere and we chose your spectral width wrongly and then you collect the digitized data in this manner and do it digital Fourier transformation then it is consequence of this digital Fourier transformation that you get lines to fold.

Let us look at that little bit more carefully, if you have an FID which is digitally collected like this and you do it digital Fourier transformation, you remember from this theorem that you get a spectrum which is given by this kind of an equation, this is

$$\frac{1}{\tau}\sum_{-\infty}^{\infty} F\left(\omega - \frac{n}{\tau}\right)$$

So, n is this is running index is the n here, now you notice we have got the series if spectra, F(ω¿ is your spectrum F(ω¿ is your spectrum and you get as many sets of such spectra as there is there are real values.

However always we chose$n = 0$, if n is equal to 0 then you have F(ω¿. So this whole range from here to here is 1 F of omega and here from here to here is another F of omega, and what is the range of this? This will be 1 by tau the distance between here to here will be $\frac{1}{\tau}$ that is

from this center to his center it will be $\frac{1}{\tau}$ because each of them is F(ω¿ is separated by 1 by tau, if n goes from 0 to 1 you increment by $\omega - \frac{1}{\tau}$ that means a whole spectrum is shifted by 1 by tau region, likewise if $n=2$ we shift it by 2 and so on.

So therefore there will be whole range of peaks, you will have spectra F(ω¿, $n=1$ will give you one spectrum, $n=0$ will give you one spectrum, $n=-2$ will give you one spectrum, -2 will be here, +2 will be here and so the whole range of frequency you will have in your digitized data Fourier transform data.

However of course we always choose one particular spectral region by using what are called as the filters. So when you do a filtering process it eliminates all the others and you pick up only this much region of the spectral width. So this shaded area here is a spectral width. Now if you have wrongly chosen your spectral width or your offset, then you may have a signal which is somewhere present here.

So this is not covered inside your spectral region, so what will happen? So this peak will appear from each of this region, from this F omega also there will be peak that comes here, the one which actually belongs to here will appear here, the one which actually belongs to this one will appear here and the one which belongs to this one here will appear here, this is an additional signal which is not covered in your spectral region.

Now therefore you will have a peak here which is coming from this spectral region n is equal to plus 1 peak will appear in this region and this is therefore called as a folded signal. Now depending upon what sort of a detection system you chose of course there will also be folding of noise, okay? So this first of all explain how the folding of signals happen? This is the consequence of digitization of your FID.

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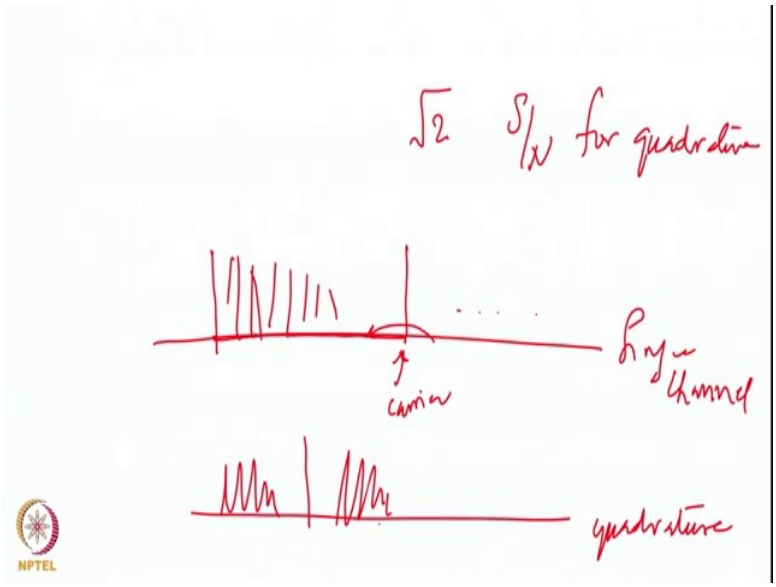

Now there will also be folding of noise, suppose your spectral region is from here to here and you put your offset in this region, suppose you put your carrier offset here and your peaks are here, okay? And this is so called the single channel detection you put the carrier at the one end of the spectrum but as a result of a Fourier transformation of course you will generate both positive and negative frequencies and those negative may appear in this area but that does not matter to us because that does not interfere with your any of your signals.

But this peaks will not fold because you have used a filter which goes like this and that will eliminate this and it will not this peaks will not matter to you but then this of course this noise which is present here which will fold in to this area, this peaks are resonated so the noise will be added into this area so the noise will be doubled up, whereas the signals are not disturbed, you will have the signals here, the signals to noise ratio will be different then what happen if you put your carrier here in the middle?

If you put your carrier in the middle, this is your region of interest and if you put the carrier in the middle the positive and the negative frequencies are distinguish in the case of quadrature detection, this is single channel and this is quadrature. So if the carrier is put here your positive and negative frequencies are distinguished, positive and negative noise will be distinguished, therefore there is no additional noise folding into a spectral region.

As a consequence if you do a quadrature detection your signal to noise will be enhance by a factor of $\sqrt{2}$, $\sqrt{2}$ enhance maintain signal to noise for quadrature, this is the consequence of folding of noise different types of holding of noise in the case of single channel and quadrature. In the case quadrature there is no folding of noise then single channel there is a

folding of noise and therefore there is loss of signal to noise factor of $\sqrt{2}$, okay. So, this is the folding problems and we are now going to see other parameters which are specific to Fourier transform NMR.

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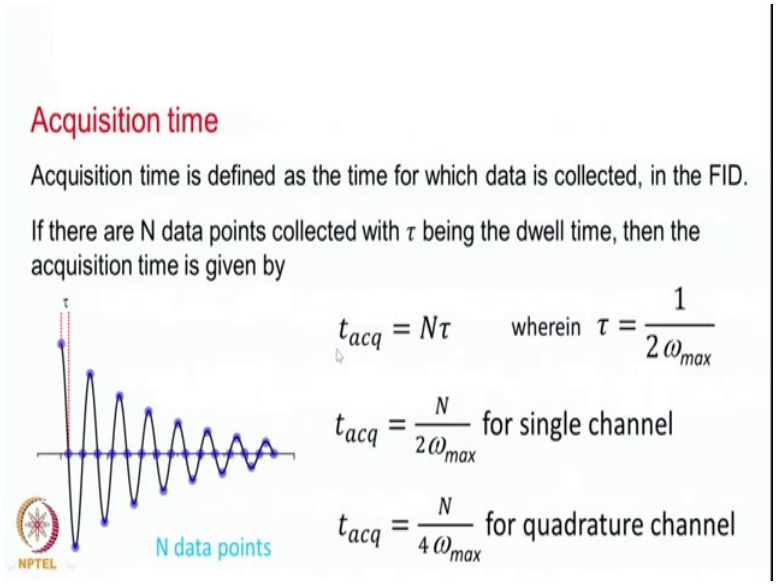

The next parameter is the acquisition time, how much data you collect acquisition time is define for which data is collected in FID. So here is FID so this goes from starts from here and you collect the data all the way up till here, right? What happens if you go beyond this? Of course there will be no signal here and there will mostly noise which is coming here. So therefore you don't want to collect noise as a typically therefore you are limited by the relaxation of the signals in as to how much data you will collect, this is the time axis here if you remember, therefore this is dictated by transfer relaxation term of your spin system.

So why about three times the transfer relaxation time, this signal would have decayed almost to 0, if you collect any data beyond that you will be basically collecting noise. Therefore, you don't want to collect any data beyond that, up till that time only you will collect. So this time is called as acquisition time, total from here to here is called as acquisition time.

Let us be more quantitative in this sense, now suppose you have collected n data points and if the time interval between two consecutive data point is τ then your total acquisition

$t_{acq} = N\tau$,

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

ω_{max} is the maximum frequency in your sampling in the spectral region.

And therefore

$$t_{acq} = \frac{N}{2\omega_{max}}$$

This is for single channel, right? In the single channel we have kept the carrier at one end of the spectrum therefore the maximum frequency is the entire spectral region spectral width.

In the case of quadrature you keep the carrier in the middle as explained to you before, therefore the spectral width is suppose it is SW, then $SW = 2\omega_{max}$ because you have put the carrier in the middle, there are positive and the negative frequencies you have omega max positive side and omega max on the negative side therefore there is a total spectral width therefore is equal to $2\omega_{max}$.

The sampling rate will be $\frac{1}{2\omega_{max}}$,so therefore your acquisition time

$$t_{acq} = \frac{N}{4\omega_{max}}$$

why is it 4 factor here? Your sampling rate is $\frac{1}{2\omega_{max}}$, yes but how many data points you collect? When you collect data you actually collect real and imaginary points in quadrature, okay? They are collected simultaneously, if you are collecting a total of N data points you collect half of them as real points and half of them as imaginary points.

So therefore you can see here if you are collecting N data points this is for the Fourier transformation and you collect $\frac{N}{2}$ points as real points and $\frac{N}{2}$ as imaginary points, that is you collect x magnetization and y magnetization, you have $\frac{N}{2}$ points for each one of those.

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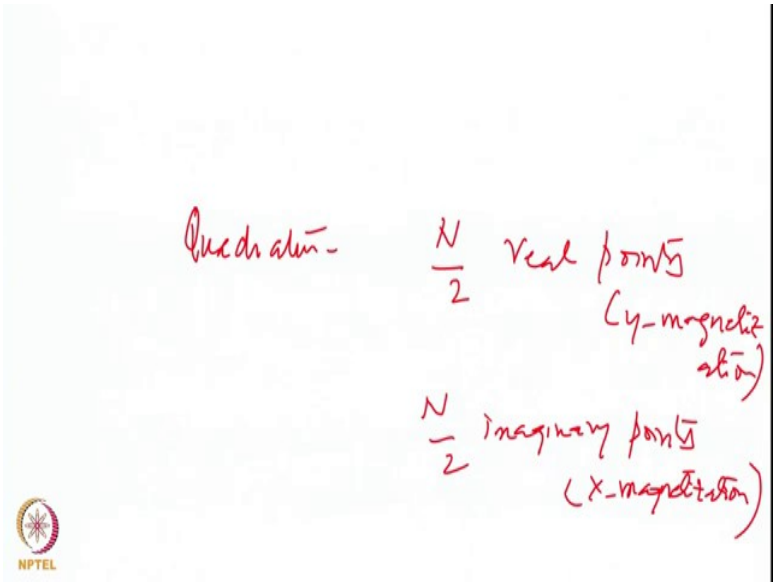

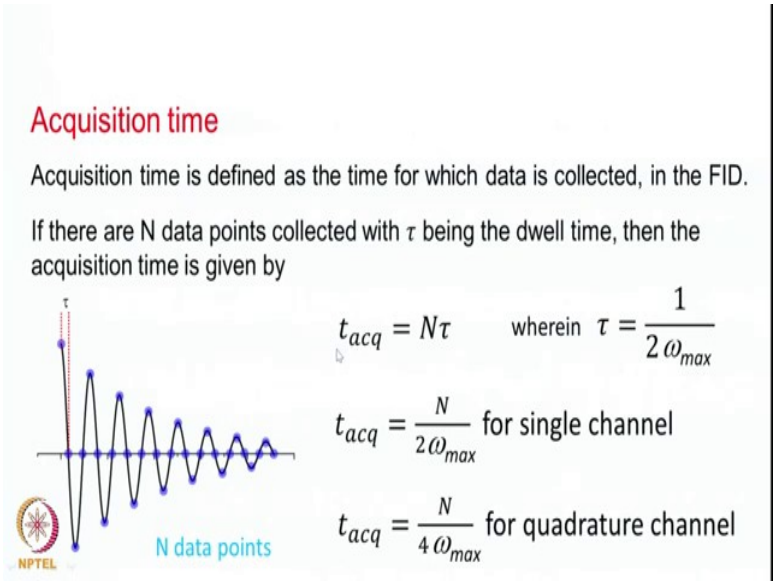

So in quadrature you collect $\frac{N}{2}$ real points that is if you called this as y magnetization and you collect $\frac{N}{2}$ imaginary points and if you called this as x magnetization, okay? So therefore your acquisition time $\frac{N}{2}$ into $\frac{N}{2\omega_{max}}$ and therefore that becomes $\frac{N}{4\omega_{max}}$, okay.

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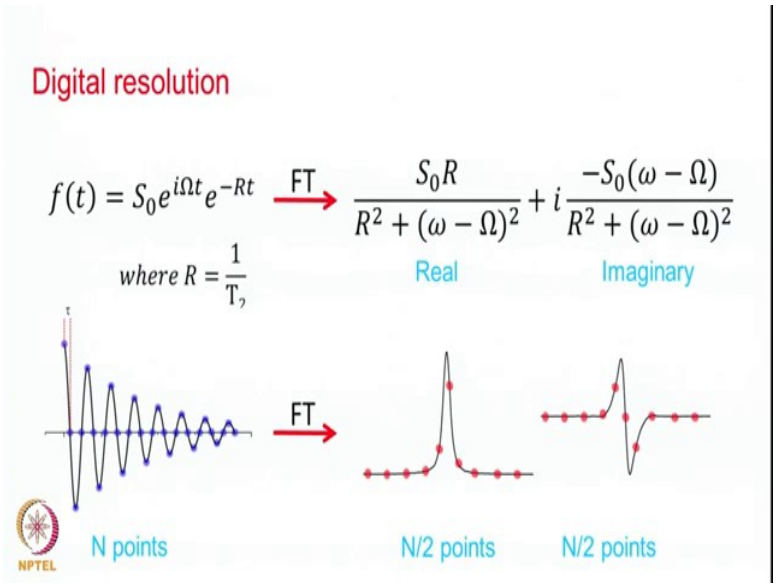

Now the next parameter that is related to this is so called digital resolution. Suppose your FID is represented by this equation

$$f(t) = S_0 e^{i\Omega t} e^{-Rt}$$

this Ω is actually your sample frequency, we collect here only let us say there is only one frequency what is the result of the Fourier transformation here?

Now you remember the spectrum also will be digital in nature, therefore there will be certain number of points in your spectrum as well, if you have collected N data points in your FID then Fourier transformation of this FID will result in two components, this is the first component is the real component and the second component is the imaginary component, this is given by this equations here and this imaginary component has results in a line shape which is like this.

And there is a results in a line shape which is like this. This is your absorptive spectrum and this is dispersive spectrum and the total N point which you have collected will now be distributed between these two spectra. So you have $\frac{N}{2}$ points to present your real data and N $\frac{N}{2}$ points to present your imaginary data and we pickup generally only the real points because it reduce levels in an absorptive line shape.

So remember both the frequency information's which are entirely present in both the real points and he imaginary points just that they have a different phase relationships, and we will be needing this at some other point later but for presentation we generally chose absorptive

line shapes which is indicated here, N data points which are collected in the FID which consist of $\frac{N}{2}$along the X axis along the Y axis and at that Fourier transformation will result in $\frac{N}{2}$ points for the real part of the spectrum and $\frac{N}{2}$ points for the imaginary part of the spectrum.

So this is the consequences of the Fourier transformation as I indicated here, you see i indicates here the imaginary component and this has the line shape which is like this, this has a line shape which is like this. And what is R? $R=\frac{1}{T_2}$, this is relaxation rate, okay? Transverse relaxations rate and that is multiplying your FID, how fast your FID is dictated by this factor and that appear as a line width in your data later on.

If this is equal to if $R=T_2$ then this is roughly will be equal to e to the minus t and then it is called as matched decay or we will see later that it is also generally used such a kind of a multiplication later on to called what is called as a match filter, that will come in a next few minutes, okay.

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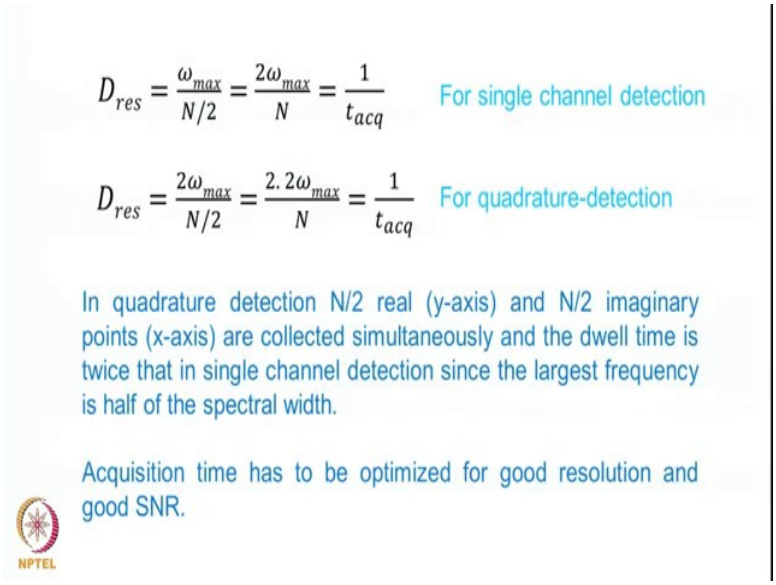

Therefore your digital resolution will be defined in this manner, you are in the single channel direction your total spectral width is

$$D_{res} = \frac{2\omega_{max}}{\frac{N}{2}} = \frac{2.2\omega_{max}}{N} = \frac{1}{t_{acq}}$$

In the case of quadrature detection also it happens in the same manner, now we have $2\omega_{max}$ is your spectral width and you are collecting $\frac{N}{2}$ data points there and therefore this is also equal to $\frac{1}{t_{acq}}$. So this is what is explained here quadrature detection, $\frac{N}{2}$real points and $\frac{N}{2}$imaginary points are collected simultaneously and dwell times is twice that at single channel at detection, since the largest frequency is half of the spectral width. So in both cases digital resolution is inversely proportional to the acquisition time and we have to optimize the acquisition time to get maximum signal to noise and maximum resolution.

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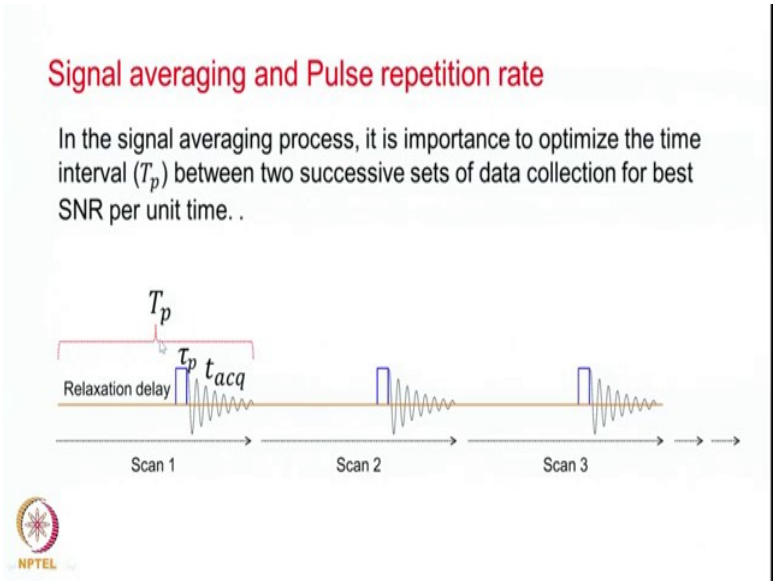

The next point to consider is signal averaging and pulse repetition rate, we say signal averaging is easy in the case of Fourier transform NMR because you can improve you collect the data for a very short period of time and you can repeat this process so you can increase your signal to noise ratio.

This is schematically indicated here, you have particular relaxation delay and you apply a pulse acquire the data and repeat this relaxation delay here again, apply the pulse acquire the data same thing continues several times. So each one of them is called as a scan, so from here it is a scan 1, here to here scan 2 and here to here scan 3 and so on and you add all these FID's these are all individuals FID's of scan 1, FID of scan 2, FID of scan 3 all these are added and you fully transform at the end, so this is the signal averaging process.

Now the question is, how much relaxation delay one should give? Because we want that magnetization should recover back to equilibrium or should it we will see in a few minutes and after that you reach a signal data collection so, what is the best value of relaxation delay one should chose or the T_p from here to here capital T_p, how much is the value of the T_p one should chose and what are the constraints in deciding on this parameter?

Digital calculations have been done because in the end we actually reach a steady state here when though the repetition the several times every scan has to be identical to the previous one and then we called the as a steady state. You it in every scan you get the same kind of the signal and then that is the steady state signal in the repetitive experiment.

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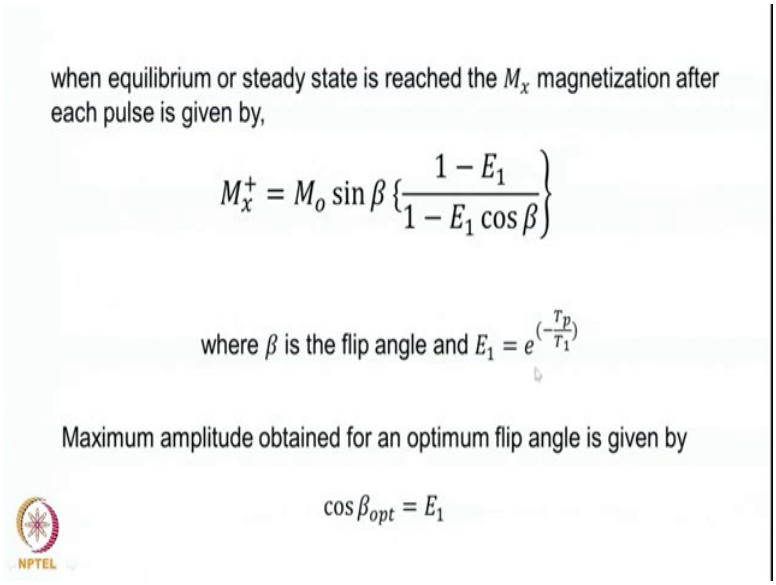

So, when you do this, one can do a calculation we will not going to the details of this calculation, this has been done when the equilibrium or a steady state is reached, the M_x magnetization after each pulse is given by this equation, this is

$M_x^{+¿} = M_0 \sin\beta \{\frac{1-E_1}{1-E_1\cos\beta}\}¿$

M_0 is the equilibrium magnetization, this is the maximum magnetization, $\sin\beta$ where β is a flip angle what you have apply, we say we have apply a pulse.

Now what should be the flip angle of the pulse? Should it be a 90˚, 30˚, 45˚what it should be? So this is the question one has to optimize, and then it is proportional to

$E = e^{\left(\frac{-T_p}{T_1}\right)}$

E1 is related to T_p and longitude in the relaxation time T_1 or the spin lattice relaxation time T_1.

Maximum amplitude is obtained for a an optimum flip angle which is given by,

$\cos\beta_{opt} = E_1$

when you match this condition you get maximum signal to noise ratio. So that is the optimization one has to do, so therefore you will see depending upon what is a value of,

$E = e^{\left(\frac{-T_p}{T_1}\right)}$

you do not necessarily have a β of 90˚, we do not necessarily required 90˚ flip angle, a detail calculation will show you what is dependence of the $\beta_{optimum}$ on the T_1 relaxation time or the ratio of $\frac{T_p}{T_1}$.

And this is indicated here, you plot here M_x/M_0as a function of flip angle, okay? For various values of $\frac{T_p}{T_1}$. So $\frac{T_p}{T_1}$ is 0.01 and $\frac{T_p}{T_1}$. is 0.1, 0.25, 0.5, 1, 2, 3, 5 and 10 and this is the 10 which you give a T_p which is 10 times T_1 value at this particular curve here and you notice for this the flip angle is 90˚. So after you apply the 90˚pulse which you have intersecting all along, you give enough time for the magnetization to recover back to the equilibrium which is along the Z axis and then you will get the maximum signal to noise ratio.

However, if the T_1 is very long then you will have to wait for a such a long time before you actually apply the pulse, so if T_1 is let us say 10 seconds then you have to wait for 100 seconds in between of two pulses in between two scans and that is actually not necessarily economical from the point of your spectrometer time or signal to noise ratios per unit time.

So, and here is a plot, for example if you are having $\frac{T_p}{T_1}$ this curve if you take this is 0.01 so if the T_1 is very long, then if you apply T_p which is $\frac{T_p}{T_1}=0.01$ then you do not need a 90˚ flip angle, you will get a maximum signal to noise ratio when the flop angle is this much only 15 degrees, okay? This is 15 degrees this may be of 10 degrees if you got this red curve $\frac{T_p}{T_1}=0.1$ then you need you get this one, you get a maximum signal when you are using approximately 30˚.

However, notice that you do not get the maximum signal here, this is the maximum signal per unit time, this is the best one we will get, okay? So therefore the signal averaging will depend upon what is it you want get for the maximum signal intensity in your spectrum, how much to optimize, okay?

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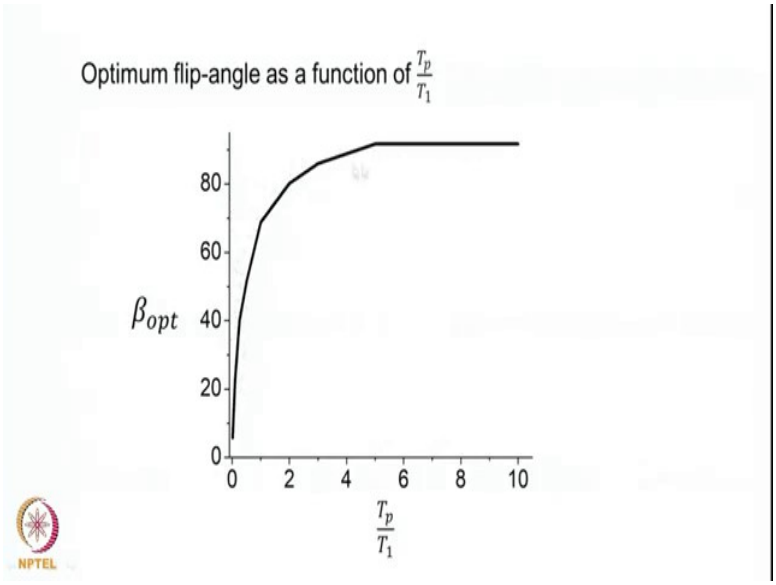

And this same curve if plotted in the different style your plot the β_{opt} verses $\frac{T_p}{T_1}$ and you can see here for very large T1's you need to have actually optimum value of flip angle is given by this manner, so you have $\frac{T_p}{T_1}$ is very small then your optimum flip angle is here and $\frac{T_p}{T_1}$ is very long then you have an 90˚ flip angle, okay?

So therefore this takes this sort of a shape in your experimental setup, this have to be optimized so that you get best signal to noise ratio per unit time in your spectrum, okay.

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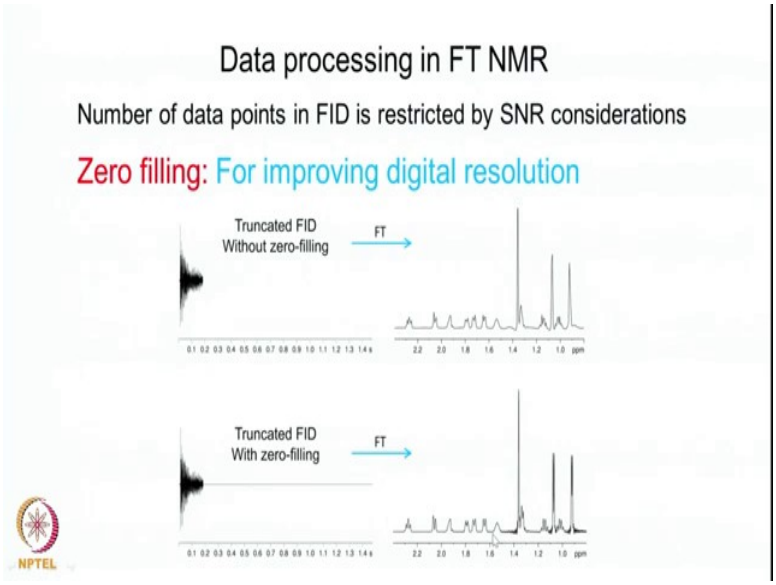

Now, the next thing is the data processing in Fourier transform NMR. The number of data points in your FID is restricted as I mentioned to you before because to signal to noise considerations, there is no point collecting data after this stage because there is only noise there, therefore you offer end up truncating your FID at this point, you have collected these many points.

Now if I do the Fourier transformation of this data this truncated FID then I get a signal like this which will have some wiggles like this, there are small small wiggles at this point and that is I mean this is not wiggles here actually this is a line shape is not very good because you have suddenly truncated FID here, you have got this problem.

Now to improve this digital resolution what one does is you add artificial points you add some additional zeroes here, this is called as zero filling, you add some additional points here this don't have a noise this are just zeroes. So instead if you collect it 1024 points here for example, you add another 1024 zeroes here, therefore your total number of points become 2048 if you add more of course you will get more points, essentially this is to improve your digital resolution not the inherent resolution.

So your number of points per hertz in you spectrum will be better, that is called digital resolution. So if you do that now if you add zeroes then your Fourier transformation then you will get the same thing but you see your digital resolution is improved, your signals are better represented in this spectrum. However, you are getting some wiggles here by the side of each signal.

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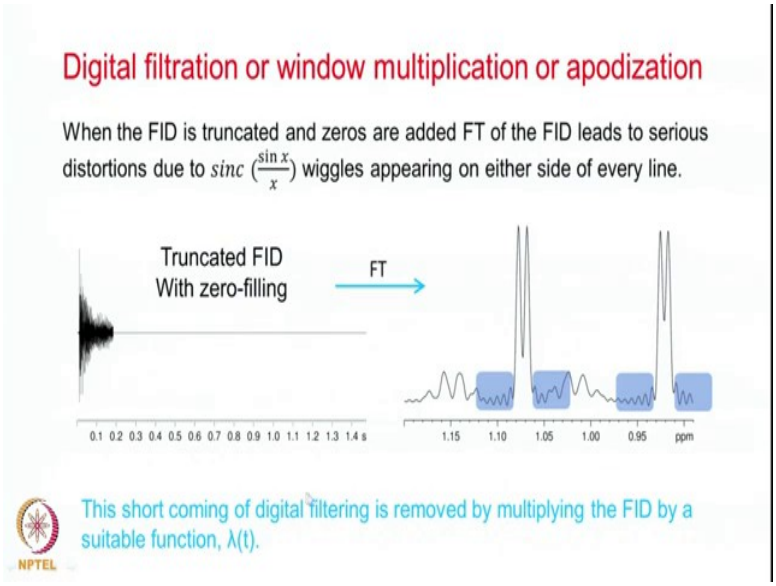

And there is more exclusively indicated here you have truncated FID with zero filling the you get this wiggles here, these are the form of sink or the $\frac{sinx}{x}$ wiggles appearing on either side of your lines and this is not desirable.

So therefore what one has to do? We have to get over this problem, we have to get over this problem and this is done by what is called as apodization or window multiplication or it is also called filtering digital filtering, okay? So there are various way one can do it, so there are various function one uses to multiply your FID, and we will show you few of those examples here.

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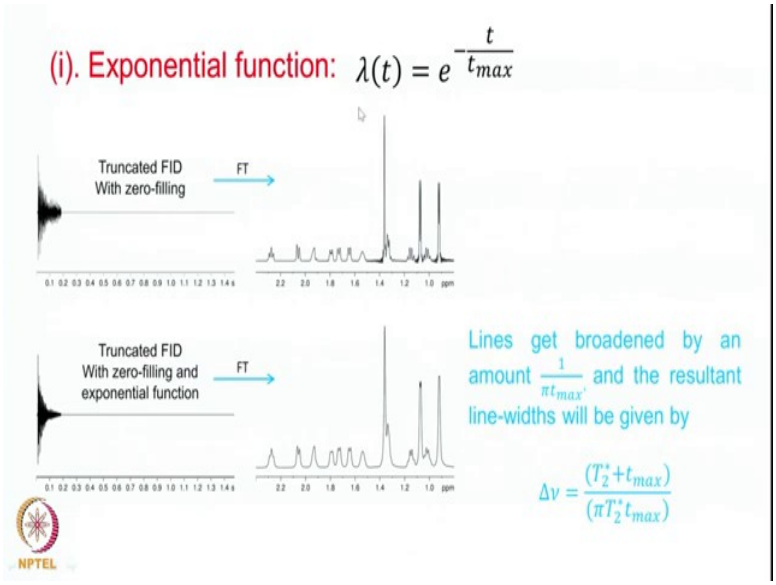

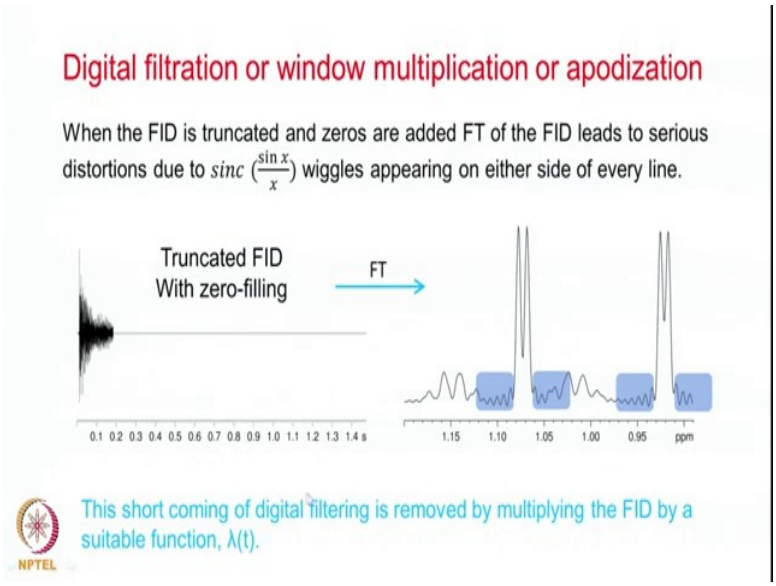

So the first thing that one could do is multiply by an exponential function which is of this form, window multiplication, digital filtration or all the three term means the same thing, okay? Different times we use different terminologies. So you multiply an exponential function, exponential function is given by this manner

$$\lambda(t) = e^{\frac{t}{t_{max}}}$$

So you have FID truncated FID with zero filling, you multiply this exponentially decaying function which goes like his this all the way till the end of the your FID and then you will get spectrum which is looking like this. So this is the result of multiplication, if you notice this certain the termination here is removed and you get smoothly going FID here and it comes 0 at this point nearly 0 and this results in a cleaner spectrum all those wiggles which were present here have vanished.

But you noticed one more thing as a consequence of this your line resolution which were present here has disappeared or rather it has reduced, so it causes the line broadening. So the lines get broaden by an amount

$$\Delta \nu = \frac{(T_2^{'} + t_{max})}{(\pi T_2^{'} t_{max})}$$

So this is the price one has to pay for improving the appearance of the spectrum in terms of the removing of the wiggles by the side of each of the lines, okay.

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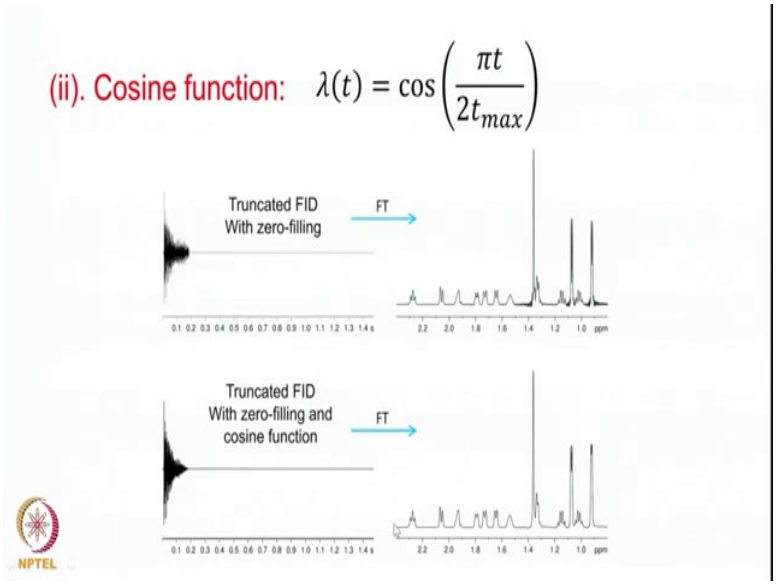

Then you have other functions, the next function which is commonly used is cosine function when you do the cosine function you multiply your FID by such a kind of a function. So this will be a function which adjusted in such a way that you start from here the 0 point is here and it will slowly come over and come to 0 at this point, okay?

So you multiply by a function essentially you apply the function with a certain frequency in other words the wave has a certain frequency or it has a certain shape so that it start at 0 here and comes to 0 at this point, so that is the last point and once you multiply with such a function then your FID becomes like this is smoothly going to 0 at this point, the wiggles has gone and also there is not much loss in your resolution in your spectrum therefore this is the optimally used multiplication and by enlarge one uses this sort of a function to improve here signal to noise ratio or digital resolution as well, okay.

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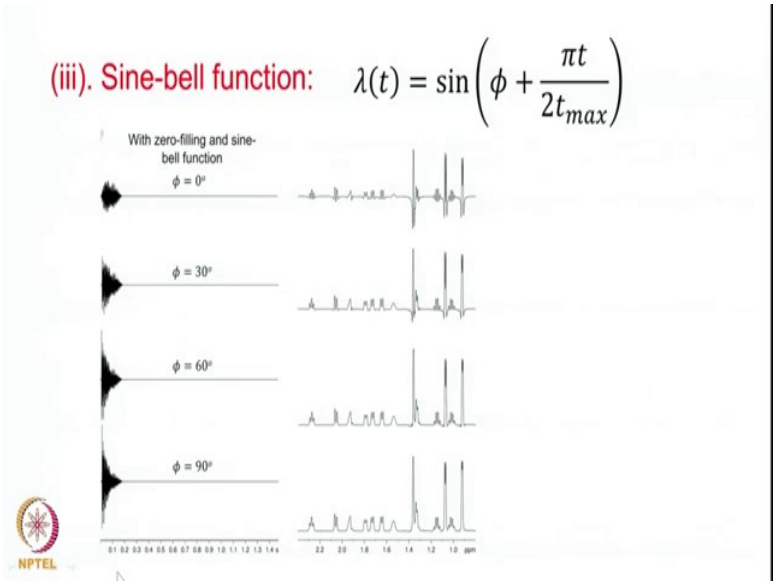

Sometimes we want to use a function of a different type which is called as a the sine bell function, the sine bell function has a this sort of a formula this is

$$\lambda(t) = \sin\left(\varphi + \frac{\pi t}{2\, t_{max}}\right)$$

This was the same as before take as the cosine but no add here a phase this is the phase, so now you see this phi if it is equal to pi by 2 this becomes the same as cosine function but putting this phase allow you different possibilities.

Now suppose you apply phi is equal to 0 and then it is a total sin function that means the sin function is 0 at time t is equal to 0 therefore this function will go like this and come down and

then comes to 0 at this point. So this is your wave this is your sine wave which is applied for multiplication.

So when you do this you get significant enhancement in the resolution in you spectrum but you also get distorted line shape, you get many negative peaks here you get each line have some kind of a wiggle like this and you have distorted line shapes, if you use phi is equal to 30 degrees then it is kind of a shifted cosine, so you start from here so it is the you don't start from 0 but you start from here so you signal to noise is sacrificed as much but you have improved resolution but still you have some of this line shape distortions you get some negative peaks which is present here.

Phi is equal to 60 produce the peaks like this and phi is equal to 90 is the same as the cosine function which were shown in the previous case.

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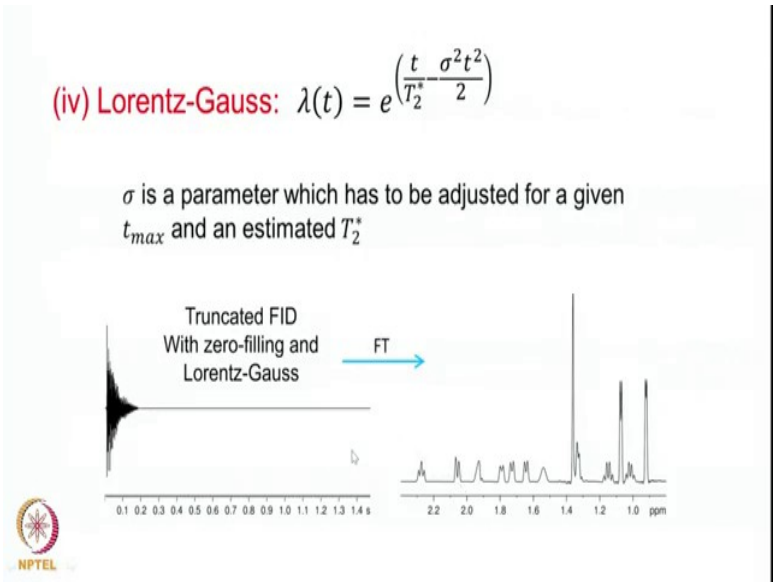

And one more function which I want to show you this is called as the Lorentz Gauss this function has this sort of a formula is

$$\lambda(t) = e^{\left(\frac{t}{T_2^*} - \frac{\sigma^2 t^2}{2}\right)}$$

σ is a parameter which has to be adjusted for a given t max and an estimated T_2^*.

So when you multiply your FID with such a kind of a function so your FID looks smoother as and it comes to 0 this point and when your Fourier transformation you have a reasonably good signal to noise ratio and resolution in your spectrum digital resolution, therefore

combining with zero fillings and multiplication by suitable functions you can get better signals to noise ration as well as better resolution in your spectrum.

So this are typical features and we may called this as cosmetics of data collection and processing and that is what is practically used and one has to optimize this parameters for better signal to noise ratio and better resolution in your spectrum, okay? So with that we come to close this session and we will continue with the data processing and other aspects of Fourier transform NMR in the next class, thank you.