

TUTORIAL

PROGRAM FID (Windows Version)

FID was written to help beginners understand the features of the pulse NMR experiment. For a given set of input parameters, which include frequencies, intensities, spin-lattice relaxation time T_1 , and the spin-spin relaxation time, T_2 , the program will display the impulse response, or free induction decay (FID). The constructive and destructive interference of the individual frequencies (actually the rotating x,y magnetization vectors) can be clearly seen. You may specify the flip angle, the receiver gain, and the relaxation delay, and to further develop the simulation you may add random noise and then watch it disappear with repetitive pulses. Exponential noise reduction may be applied. In the jargon of the FTNMR experiment, this is called "line broadening." Somewhat the opposite manipulation, resolution enhancement via a Lorentz-Gauss transformation may also be performed. Another operation allowed is apodization. Finally, sampling rate and aliasing may be addressed. You choose the sampling frequency and the program marks the points on the FID where data are taken. In this way it is easy to see that sampling frequencies that are too low will lead to transformed spectra with erroneous frequencies.

After observing the FID, the Fourier transform may be calculated and the frequency domain spectrum drawn. Examination of the transformed data shows clearly how noise, signal averaging, receiver gain, T_2 , T_1 , sampling time, and sampling rate influence the appearance of the spectrum. Problems with phasing are demonstrated by introducing a time delay before acquisition of data. These spectra may be "phased", and there is even a primitive baseline flattening algorithm in case there is an uneven baseline. Saturation effects are demonstrated by having too short a relaxation delay for nuclei with large T_1 .

The simulations are reasonably authentic. The major limitation is in the allowed frequency range. In an actual proton or carbon experiment you might have the irradiating frequency separated by 1-10 KHz from the frequencies being observed. In our simulation the screen resolution limits the frequency spread to about 50 Hz if you want a good view of your FID. If you really aren't interested in the appearance of the FID, then frequencies as large as 1000 Hz are o.k. Also, we can't do Fourier transforms larger than 4096 points, and this somewhat limits the frequency range/resolution of the simulation.

FID for Windows

GETTING STARTED

If the program has been installed, you simply click on the FID icon. If the icon is not found, you could look for FIDWIN.exe. If this is not present, you will need to install the program. Run program SETUP.exe (found on the FIDWIN disk) and a program icon will be created.

If you have a HP plotter or printer attached, you can make high quality copies of the spectra that appear on the screen, but this is not required for today's session. A laser printer is fastest, and an ink jet is ok, but you miss the pretty colors provided by the HP plotter. A calculator and ruler would be helpful for this session.

The startup window offers four options. You may choose: 1) to work with the frequencies that give the FID that is shown on the screen, 2) to input frequencies of your choice, 3) actual proton systems, or 4) actual carbon systems. Later you will want to examine options three and four, but for these lessons you will be working with frequencies of your choice, so please select option two and click on the continue button. Now you will see a window asking if you want to input 1-5 single frequencies, or the frequencies corresponding to a spin-spin splitting pattern. If you select 1-5 single frequencies you will be asked to enter them, along with the corresponding intensities. If you select coupled systems, then you will need to enter chemical shifts and coupling constants. The program will calculate the frequencies for you. Please check the option that calls for entry of 1-5 single frequencies.

You should click the help button on this window and note the limitations on input. We will summarize a little of it here. Frequencies are input in Hz, and can be positive or negative. Intensities are input in inches, as is noise on the next window. If you input a single frequency of 1 inch, you will get an FID that is 1 inch tall at time = 0. (assuming a 90° pulse, receiver gain = 1, and no saturation of signals) An intensity of 0 is ok. You might do this if you want to look at noise only. T_1 input is optional. Leave all T_1 s = 0 if you want the simulation to ignore the effect of signal saturation when repetitive pulsing is carried out.

The remaining input comes on the next window. Default values are supplied, but we will be asking you to change many of these in this tutorial. If you look at this window you will see that provision is made for noise, flip angle, repetitive pulses (number of pulses and relaxation delay), data acquisition (number of data points and spectrum width), acquisition delay, receiver gain, T_2 , and type of detection. It would be a good idea to click on the help button and read what is written about each of these parameters. Just in case you don't, here is some info.

Noise (inches)

For simulating noisy spectra (0.0-3.0). Noise is specified in inches. The intensity of the signal, in inches, will be the **sum** of the intensities that are input. Suppose you input one inch of noise, and three frequencies, each with an intensity of 1. The signal at $t=0$ would be 3 inches tall, if a 90 degree pulse was used, and the noise could possibly contribute another inch. If a smaller flip angle is used, the signal will diminish, but the noise will remain constant.

Flip angle

May be placed anywhere between 0 and 90°.

Spin-spin relaxation time (T_2)

A "good" magnet is simulated with a value of 1-2 sec. A value of $<.5$ sec would simulate poor field homogeneity.

Receiver gain (0.01-100)

Used to change size of FID. The "receiver" has room for 3 inches of signal, to either side of zero. Anything above 3 inches will be lost. So if you have lots of noise and lots of signal, you might need to set the receiver gain below 1. Alternately you could use a smaller pulse.

Acquisition delay (sec)

Use nonzero value to show phasing problems in transformed spectra (0.000-0.050 sec).

Number of repetitions

Has two uses in this program. First, allows simulation of signal averaging of noisy spectra. Second, allows demonstration of saturation when sample is pulsed repetitively before it has a chance to relax. Allowed values are 1 and 10-500. If repetitive scanning is to be done, and if T_1 effects are to be taken into consideration, you need to ask for at least 10 repetitions. The equation used to calculate the contributions of the individual nuclei to the total FID assumes a steady-state condition, and this requires about 10 repetitions to be valid.

Relaxation delay (seconds)

Use to set the time between pulses in the repetitive scan mode (≥ 0). Pulse interval = relaxation delay + acquisition time. Meaningless if only one pulse is used. Also, will not have any effect unless you set T_1 values.

Spectrum width (Hz)

Should be made larger than the largest frequency present to avoid aliasing. Display is 8 inches wide, so widths divisible by either 4 or 8 will make better-looking plots. The sampling rate will be made twice the spectrum width. For example, setting the width at 100 Hz will cause the sampling rate to be 200 points/second.

Number of points

In the Fourier transform the allowed values are 128, 256, 512, 1024, 2048, and 4096. The number of points, and the sampling rate discussed above, will control the acquisition time:

$$\text{acquisition time} = \text{number of points} / \text{sampling rate}.$$

Mode of detection

Quadrature detection allows for the input of positive and negative frequencies. If you choose quadrature detection you actually are using **twice** the number of points as indicated in the preceding paragraph, and the spectrum width will actually be **twice** the value you input. Bad quadrature will give some false peaks, but they will be low in intensity. No quadrature (the way we used to run NMRs) will give spectra whose positive and negative frequencies fold into each other. Ordinary quad detection is recommended for these exercises.

Once you proceed from this window you will be shown the FID that corresponds to the data that was input. The menubar that appears will allow you to 1) view different portions of either the real or imaginary parts of the FID, with optional viewing of the individual frequencies as rotating x,y-magnetization vectors. 2) view the individual frequencies superimposed on the FID., 3) view the datapoints acquired by the analog-to-digital converter during data acquisition. These points can be viewed either with, or without, having the complete FID superimposed. Provision is made to allow curvefitting of sin and cosine waves to the imaginary and real points if only one frequency is entered. This is useful in learning about aliasing (Lesson 11). Other options include quitting, going back, and going ahead with the Fourier transform. At the time you click on "continue" you will be asked to indicate if you wish to zero fill.

When you give the go-ahead to proceed toward a transformed spectrum you will get another menubar that will afford you the opportunity to do some post-acquisition data processing before transformation to the frequency domain. Allowed processing includes exponential smoothing, resolution enhancement, and apodization (only if you zero fill). It should be noted that the FID is saved, so if you do some post-processing, and you don't like the results, you may recover the original FID and try something else.

Upon asking for the Fourier transform, the complete frequency domain spectrum will appear on the screen, along with a menubar that allows for new views (reset zero reference, change x-axis limits, change y-axis scale), phasing, integrating, hard copy production, starting over, and quitting. If you set the acquisition delay >0 , you will probably want to phase the spectrum. When you click the phase bar you will be asked whether you want to remember the phasing of the last run, or start phasing from scratch. Once you respond, you will get the phase menubar with options listed for positive and negative phase corrections, and the amount of the correction (3, 10, 25, 180, & 360°). Once you have finished you will be asked if you want to smooth the baseline. This is probably not important unless you wish to integrate the spectrum. Please note that to get to the baseline smoothing routine you must first phase the spectrum. Even if the spectrum doesn't need phasing, you still must do it if you want to spruce up the baseline before integrating. Don't forget!

That's enough for now. We'll talk more about all of this as we go through the lessons that follow.

SINGLE-FREQUENCY SIMULATIONS

- 1) **What does an FID look like? What is the effect of T_2 ?** Let's start by asking for a single frequency, 5 Hz, with intensity=1. Do not ask for T_1 input (i.e., make it 0). The parameters on the second window should be set as below:

Noise	0	Flip angle	90
T_2	2	Receiver gain	1
Acquisition delay	0	# of Pulses	1
Relaxation delay	0	Spectrum width	64
Quad detect		Points	256

The screen display should show 2 sec of a decaying cosine curve with a frequency equal to your input value. Check to see that this is so. The intensity of the signal as a function of time is given by the equation $I_t = I_0 e^{-t/T_2}$. If you used a T_2 of 2 seconds, then this equation predicts that after a time of 2 sec, the signal remaining will be 35% of the original signal. Measure it with a ruler. Using the same T_2 value as before, calculate the signal remaining after 6 sec. If you used a two second T_2 , you should find that only 5% of the initial signal remains after 6 seconds. Think about this. Would it do much good to collect data much past 6 sec for this sample? Wouldn't you be collecting mostly noise? Click on <Start Over> to go back to the parameters window. Change the number of points to 1024; this will increase the acquisition time to 8 seconds. The plot will appear as soon as you click on the <Continue> button. Look at the intensity at 6, 7, and 8 sec. Not much there?

- 2) **What happens to the FID if the signal relaxes faster?** Go back to the parameters window and change T_2 to 1 sec and find out. Notice the greater rate of signal disappearance. How much signal remains after 2 seconds? Does the display agree with the equation? Make a note of the frequency you used; we will use it several more times.
- 3) **FIDs at other frequencies?** Click on <Start Over> and choose the complete restart option. Make the frequency either larger or smaller and leave everything else the same as in #2 above. The rate of signal disappearance should be independent of the frequency used. Is it?

- 4) **Try a puny pulse; then enlarge the signal with more receiver gain.** Repeat your very last simulation, changing the flip angle to 20°. This puts less of the z-magnetization into the x,y plane, and as a result the signal is weaker. Click on <continue> to see the FID; it should be less intense. Since we haven't introduced noise yet, this operation is perhaps fraudulent, but let's do it anyhow. For the next simulation, leave everything unchanged but the receiver gain. Increase it to about 3. You should find that you are back to nearly the original signal intensity. However, if noise had been present, it, too, would be amplified. What would happen with a really large receiver gain. Take it all the way to 30 and see. You should see an FID that is chopped off at small time values, and as you might suspect, this is not good. A FT on this truncated FID will have some peculiar features, as you will presently see!
- 5) **Noise is a problem in FTNMR.** Use the same single frequency you have been using, but this time add 0.2 inch of noise. In case you have lost track of some of your input variables, we show the parameter window below.

Noise	0.2	Flip angle	90
T ₂	1	Receiver gain	1
Acquisition delay	0	# of Pulses	1
Relaxation delay	0	Spectrum width	64
Quad detection		Points	1024

Notice at time=0, where the signal is approximately 1 inch tall, that this noise is not too noticeable, but at times >3 the noise becomes more pronounced. **This noise will be carried over into the transformed spectrum.** Click on the <Cont> menubar and <Dont Zero Fill>. When the next menubar appears, click on <Do FT Now>. The transformed spectrum is noisy; we really shouldn't have acquired all that noise after about 4 seconds! Let's look more closely at the spectrum. Draw horizontal lines across the top and bottom of the noise. Measure the distance, n, between these lines. Also measure the distance, p, from the peak maximum to the middle of the noise. The s/n ratio is given by the equation $2.5 \cdot p/n$. Make a note of this value. Now, repeat the above run, changing the number of points from 1024 to 512. This will halve the acquisition time, thus affording a better match between the time at which the data becomes worthless and the time at which we stop acquiring. Proceed to the transformed spectrum and measure s/n as above. You should see a decrease in noise, and thus an increase in s/n.

- 6) **Noise is random. It can be reduced by adding several FIDs before performing the transform.** Repeat the 1024 point simulation in 5, with everything the same except this time ask for 16 pulses. Before showing you the final FID, the program will show you the FID after 1 and 10 pulses, so it is easy to see how the noise diminishes with repetitive sampling, or

signal averaging. As before, skip over line broadening and resolution enhancement and observe the transformed spectrum. The s/n ratio should increase with the square root of the number of scans. Since you did 16 times as many scans as in experiment 5, the s/n should increase by a factor of 4. Does it?

- 7) **Exponential smoothing saves instrument time, but for a price.** Suppose you don't have time to make the necessary number of repetitions. You might try exponential smoothing, or "line broadening". The FID at all times t is multiplied by e^{-nt} , where n is the line broadening factor (usually <2). In the initial stages of the FID, where t is small, this multiplication doesn't change the impulse response very much, but as t becomes larger in the later stages of the FID, the exponential multiplication attenuates the signal and the noise. The attenuation of the noise results in a less noisy spectrum, but the loss of signal at longer times t unfortunately produces line broadening. To see this for yourself, set the parameters as you see them below.

FREQ: 15 INT: 1 T1: 0

Noise	.2	Flip angle	90
T_2	1	Receiver gain	1
Acquisition delay	0	# of Pulses	1
Relaxation delay	0	Spectrum width	50
Quad detect		Points	512

After the FID is displayed, click on <Cont>, then <Dont Zero Fill>. On the next menubar, click on <Exp. Smoothing>. Accept the default line broadening factor, n , of 0.5. The envelope of the filtered FID will be superimposed on the original FID. At this point you may accept this value or keep trying until you get one that you like. Once you accept a value, ask to see the FID with the filtering. Notice how much "better" it looks after filtering, but remember that if you make the signal attenuate with time, broader peaks are unfortunately the result. The transformed spectrum clearly shows this; ask for the transform and see for yourself. Try one or two other line broadening values before continuing. Notice how the broadening increases and the noise decreases as you make n larger.

- 8) **Collecting data for longer times leads to sharper peaks, up to a point.** In this series of experiments we will explore the impact of the number of points in the Fourier transform. Use a frequency of 10 Hz, and omit setting T_1 . Make $T_2 = 4$ sec., receiver gain = 1, noise = 0, a single 90° pulse, and a spectrum width of 16. Do a 128 point transform, then 256, and finally 512, without any zero filling, acquisition delay, line broadening, or resolution enhancement. You should see the linewidth diminish as more points are used. The reason is with more points, we are able to collect data for a longer time. This should lead to sharper peaks (seen better with an 8-12 Hz expanded display). But will it work if T_2 is only 1 sec?

Here the signal will quickly disappear, so collecting data for long times won't help, for there is nothing there but noise. Set the relaxation time to 1 second and repeat this sequence to see for yourself.

- 9) **Making something out of nothing, i.e. zero filling.** Create a system with a single frequency at 10 Hz, an intensity of 2, and a T_1 of 0. Have the parameters window as shown below:

Noise	.1	Flip angle	90
T_2	1	Receiver gain	1
Acquisition delay	0	# of Pulses	1
Relaxation delay	0	Spectrum width	24
Quad detection		Points	256

With these settings, data will be collected at a rate of 48 points per second, and the acquisition time will be 5.3 sec. (256/48). This is about right for a system with a T_2 of 1 sec., for there isn't much signal after 5 seconds anyhow. Do the transform without any line broadening or resolution enhancement, and make an expanded scale plot of the spectrum (6-14 Hz), and if possible, get a hard copy. If you don't get a hard copy, study the peak carefully. This transformed spectrum has 256 digital points to define 48 Hz (24 to either side of zero), which means that the resolution is 0.19 Hz/point. Suppose you want more resolution. What do you do? One possibility is to ask for a 512 point transform. (Do it, and compare the transformed spectrum with the one above.) With twice as many points as before, the resolution would be 0.095 Hz/point, and the acquisition time would be 10.6 seconds. But during the last 5-6 seconds of the FID, there is no signal, only noise. Rather than add this much noise to the FID, you can get the desired improvement in resolution by simply adding 256 zeros! Perhaps the word resolution is not the most appropriate. What we're doing is defining the curve (peak) with more points, so it looks smoother. This is kind-of like interpolating once between every set of adjacent points. Change the number of points back to 256, ask for zero filling and run the simulation. Do not ask for line broadening, resolution enhancement, or apodization. Compare the transformed spectrum with the two above. You should find that the zero filled spectrum is a little better looking than the 256 point original spectrum in this series, and just as good as the 512 point run.

- 10) **Don't use a spectrum width that is too large, or your lines will broaden.** Use a single frequency, perhaps near 10 Hz. On the parameters window set T_2 to 2 sec., noise = 0, and use one 90° pulse. Do a series of 512 point transforms with no zero fill, varying the spectrum width from 24 to 48 to 96 Hz. To display 96 Hz, the FID must be sampled 192 times/sec., so the acquisition time is only about 2.7 sec. That's not enough for sharp lines. In contrast, a 24 Hz spectrum width requires only 48 points/sec., so data acquisition can continue for 10.6

sec. Do these three transforms without any line broadening or resolution enhancement, and display an expanded plot, perhaps from 6-14 Hz for each. You can then see the broadening of the line as you proceed from 24 to a 96 Hz spectrum width.

So what must we do to get the sharpest peaks? Answer - acquire data for the longest possible time following the pulse, but for this to work you need long T_2 s (well-tuned instrument) or the signal will disappear too soon. Transform size must be large, or there won't be any place to put the data. Finally, make the spectrum width as small as possible. Large spectrum widths require large data acquisition rates, and this will use up your computer memory in the early stages of the FID and you'll have no place to put the later data.

- 11) **A spectrum width that is set too small really makes a mess of things.** To see how this happens, use a single frequency of 3 Hz, an intensity of 2, and $T_1 = 0$. Have the parameters window read as follows:

Noise	0	Flip angle	90
T_2	2	Receiver gain	1
Acquisition delay	0	# of Pulses	1
Relaxation delay	0	Spectrum width	2
Quad detection		Points	128

This should produce a spectrum with a peak at -1 Hz, not 3 Hz! This is the folding, or "aliasing", phenomenon - a very undesirable consequence of setting the spectrum width too small. How can this happen? Repeat the simulation, but this time ask to see the FID expanded to show the first 0.8 seconds (real). Then ask to see the data acquisition points without the FID. The computer acquires data at a rate which is exactly twice the value of the specified spectrum width, so in this run you should see four points per second on the FID. Now four points per second is not enough for the proper definition of a cosine wave with a frequency of 3 Hz. (You'd need at least 6/sec. to do the job.) A window should appear on the screen that asks you if you would like to curvefit the points. Answer yes, but before doing so, enter 3 Hz as the starting frequency for the curvefit. You will notice that the 3 Hz wave fits the points, but it is not the **lowest** frequency that fits. Depress the <down arrow> button and lower the frequency to 1 Hz. Notice the fit? Now take the frequency down to -1 Hz; you should see that it, too, fits. Repeat the entire procedure with an 0.8 sec display of the imaginary points. This time, you should find that only the -1 Hz wave fits the points, not the +1. If you find this stuff interesting, you could start over and **not** use quad detection. There is no imaginary signal; in the real domain, both +1 and -1 Hz will fit, and if you look at the transformed spectrum you should indeed find both peaks present.

- 12) **Truncated FIDs give distorted spectra. Don't turn up the volume too much!** Enter parameters as shown below.

FREQ: 15 INT: 1 T1: 0

Noise	0	Flip angle	90
T ₂	1.5	Receiver gain	10
Acquisition delay	0	# of Pulses	1
Relaxation delay	0	Spectrum width	32
		Points	512

The important item in this list is the receiver gain of 10. This simulates sending too strong a signal to instrument's a/d converter. You should see an FID that is very truncated at early times. By asking for a display of the acquisition points you can see that the data going to the computer doesn't look at all like a normal cosine wave below t=2 sec. Proceed with the Fourier transform; notice the extraneous peaks? Clearly this is to be avoided when you run actual spectra. Some of the better NMR software will adjust the gain automatically. Isn't it nice that they do that for you!

Truncation at the end of the FID can also cause problems in transformed spectra. To demonstrate this we will need to input a larger T₂ value and a larger spectrum width and/or fewer points in the transform. This will give us an FID which does not decay before our last data point has been acquired. Here are suggested inputs:

Frequency window: FOUR freq, spread between 10 & 80 Hz, all inten = 1, & T₁ = 0.

Parameters window:

Noise	0	Flip angle	90
T ₂	3	Receiver gain	.6
Acquisition delay	0	# of Pulses	1
Relaxation delay	0	Spectrum width	96
		Points	512

Proceed to the Fourier transform, and ask for zero filling when prompted. Do not ask for line broadening or resolution enhancement, and do not ask for apodization yet. We'll do that next. Also, don't worry about the way the FID looks, we'll talk about that later in the section entitled "More Than One Frequency." These parameters dictate that data is taken for only 2.7 seconds, but the FID is still intense at that time. As a result there is a big discontinuity between the intensity of the last points taken from the FID and the 512 zeros added. The transformed spectrum should again show extraneous peaks which appear as wiggles to either side of the peaks. Expand the region near one of the peaks to see this better.

These wiggles can be removed by apodization. In this operation the FID is tapered to zero at the end of the acquisition period. In our algorithm you specify the number of seconds over which the tapering takes place. The envelope of the FID will be displayed before and after apodization. You may either accept the last apodization time and proceed to the FT, or you may ask for a new time before proceeding. Apodization will broaden the peaks somewhat. Repeat the above run, with about one second of apodization and see what happens. The exponential smoothing operation discussed earlier is considered by some to be an apodization, carried out over the entire FID rather than the last second or so. You might repeat this run, this time choosing enough line broadening to bring the FID near zero at 2.7 seconds and compare the transformed spectrum with the ones just done.

- 13) **Transformed peaks sometimes appear upside down, or worse. The unavoidable phase problem.** So far we have assumed that data acquisition commences immediately after the pulse. In the actual nmr experiment, this is not possible. There is always a short time delay after the pulse, before data acquisition is begun. In this simulation we will show you how this affects the transformed spectrum. The suggested parameters are shown below:

```
FREQ: 15  INT: 1  T1: 0
      Noise          .05  Flip angle      90
      T2            1    Receiver gain  1
      Acquisition delay .01 # of Pulses    1
      Relaxation delay  0    Spectrum width 32
                               Points 256
```

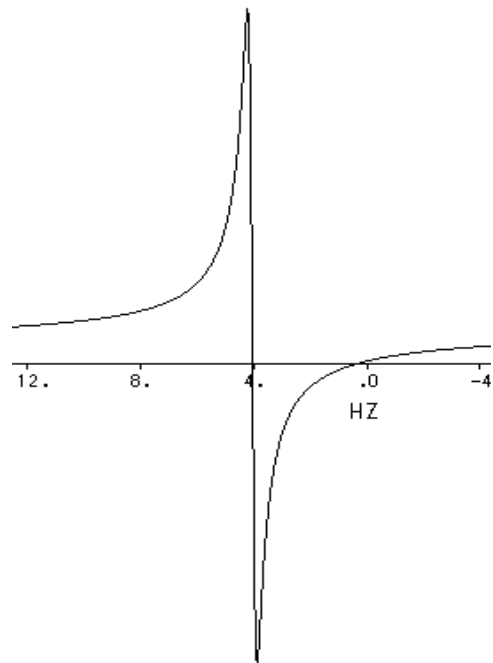
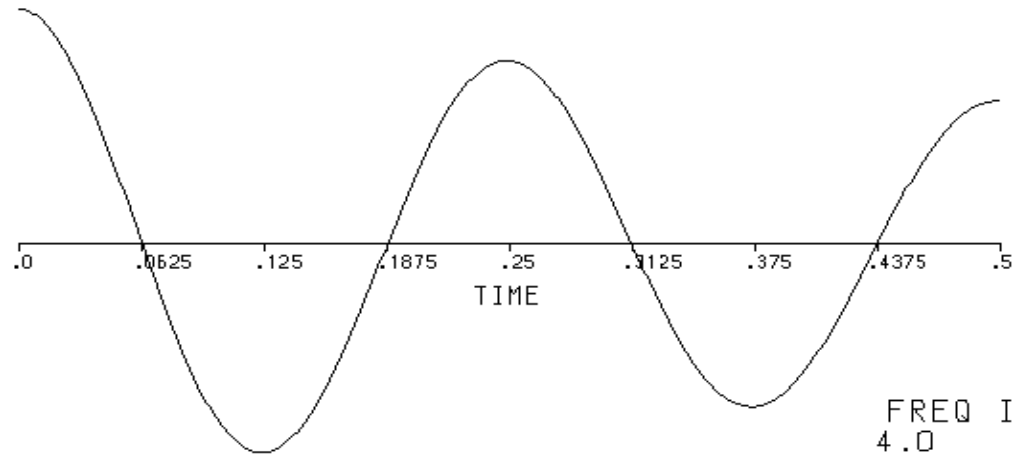
The acquisition points shown in the FID still properly define the frequency of the signal you input, but the first point taken does not coincide with time = 0. Perform the transform without zero filling, line broadening, or resolution enhancement. Notice that the peak is out of phase. Depending on the frequency you input, the acquisition delay chosen, and the spectrum width, you could see a peak that needs a phase correction anywhere between 0 and 360°. On the next page are shown three spectra which need "phasing". The first needs a correction of -90°. You will get this pattern if you start acquiring data where the FID crosses zero from positive to negative (0.0625 or .3125 sec in this example). The second is 180° out of phase and it would be seen if data collection commences in a "trough" (0.125 or 0.375 sec here). Finally, the third spectrum is 90° out of phase; you will see this pattern if data collection begins where the FID crosses zero from negative to positive (0.1875 or 0.4375 sec for this 4 Hz wave). Data acquisition beginning at the "top" of the wave (here at t=0.0, 0.25, or 0.5 sec) will give a transformed spectrum that needs no phase correction. One final comment: When you have a spectrum with several frequencies, it would be quite a coincidence if the same phase correction applied to all.

Now try to phase the spectrum on the screen. You begin by marking the peak to be phased. The amount of the phase correction will be 25° unless you change it by clicking on the <amount> menubar. Clicking the <+> menubar will result in a $+25^\circ$ correction; clicking the <-> menubar will result in the application of a -25° correction. The amount of the correction is varied in a linear manner across the spectrum, with a peak at $\nu = 0$ receiving a zero correction.

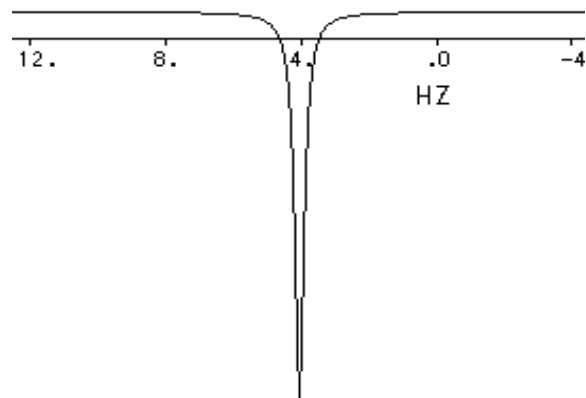
- 14) **Baselines in FTNMR can be uneven. This causes trouble with integrals.** Once you indicate that you have finished the phase correction you will be asked if you want to smooth the baseline. Answer no the first time; then ask for an integration. If the baseline is uniform, but above or below zero, the integral routine has a leveling function that will work. However, if the baseline has a little roll, the integral will look awful. Repeat the run, and this time do the baseline smoothing before integrating. The integral should look better.

Before moving on to the second part of the lesson, which deals with FIDs containing more than one frequency, you might consider some runs with bad quad detection, or no quad detection at all. Also, if you haven't looked at our rotating vector show, you might do so now. Create a system with a 20 Hz FID, using a spectrum width of 40 Hz, with 256 points. Click on the left-hand menu item <Show FID>, and then <Show Vectors>. The rotating X,Y magnetization will be shown concurrently with the FID evolution. You can pause the show at any time by depressing the space bar.

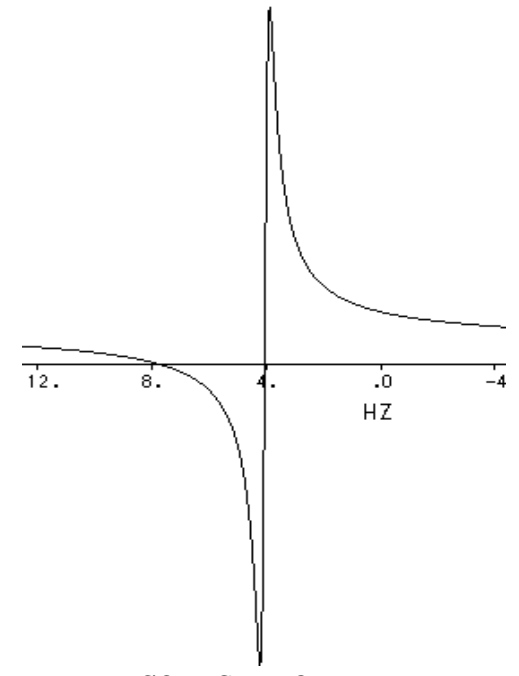
PHASING PROBLEMS CORRESPONDING TO DATA ACQUISITION BEGINNING AT TIMES > 0.0



DATA ACQUISITION COMMENCING AT 0.0625 SEC.



DATA ACQUISITION COMMENCING AT 0.125 SEC



DATA ACQUISITION COMMENCING AT 0.1875 SEC

MORE THAN ONE FREQUENCY

For the remaining simulations we will look at FIDs containing 2-3 frequencies (the program can do 5). You will learn how the FID is influenced by frequency, intensity, and T_1 when more than one frequency is present. Finally, you will learn about resolution enhancement.

- 15) **FIDs that contain more than one frequency give complex patterns.** We will begin with a simple case - two frequencies, perhaps 5 and 6 Hz, with equal intensities, and no T_1 provision. Don't ask for a Fourier transform yet; just look at the FID. Choose any value of T_2 , leave out the noise, and set the receiver gain to 1. Make the spectrum width 16 Hz and the number of points = 128. You will notice that the FID is more than a simple decaying cosine curve, because the two frequencies constructively and destructively interfere with each other. If you want to better see how this happens, ask to see the individual frequencies superimposed on the FID. If things appear cluttered, ask for an 0.8 second expansion.
- 16) **The same compound can produce two very different-looking FIDs!** How can this be? Suppose we have a sample with two frequencies, perhaps TMS and one additional peak, separated by 4 Hz. For this simulation use frequencies of 3 and 7 Hz, $T_2 = 2$, no noise, one 90° pulse, a spectrum width of 32 Hz, and a 256 point transform. This would correspond to our hypothetical compound plus TMS, with the irradiating frequency set 3 Hz below TMS. Make a mental note, or a hard copy, of the FID. Then change the frequencies to 23 and 27, keeping everything else the same. This is the same compound; the only difference is that we have moved the irradiating frequency. Notice how different the FID appears, yet the transformed spectrum shows two peaks, separated by 4 Hz. as before.
- 17) **Small peaks can get lost in a spectrum that also contains large peaks and a little noise. The DYNAMIC RANGE problem.** Create a system with two, or more, frequencies of unequal intensities. First use two frequencies, perhaps 10 and 15 Hz, and make one or the other progressively more intense until you reach an intensity ratio of 99:1. The parameters window should be as shown below:

Noise	.03	Flip angle	90
T_2	1	Receiver gain	1 (or less)
Acquisition delay	0	# of Pulses	1
Relaxation delay	0	Spectrum width	32
Quad detect		Points	256

Display the first 0.8 sec of the FID and show the individual frequencies. You should notice that near 30:1, the FID is primarily that expected for the single intense frequency; indeed you may not notice any difference at all from a single-frequency FID. The transformed spectrum (256 points, 32 Hz width) may still show the weaker peak, but poorly. If you add more noise and possibly a little line broadening, the weak peak will disappear entirely. The question of dynamic range is important, particularly for those who wish to analyze mixtures. In the real world, dynamic range is a function of the word length of the computer and the receiver gain of the signal going into the a/d converter.

- 18) **You can't always trust peak area measurements. Slow-relaxing signals, those with large T_1 s, sometimes nearly disappear.** When you have two frequencies with different spin-lattice relaxation times, you will probably have trouble with your peak integrations. This is common with carbon-13, but not protons. Enter two frequencies, perhaps 10 and 15 Hz, with equal intensities, one with a T_1 of 3 sec and the other 15. Make the parameters window as shown below:

Noise	.03	Flip angle	90
T_2	1	Receiver gain	1
Acquisition delay	0	# of Pulses	1
Relaxation delay	0	Spectrum width	32
Quad detect		Points	256

Proceed with the Fourier transform, but don't zero fill, line broaden, or resolution enhance. The transformed spectrum should show two peaks of nearly equal intensities. Theoretically they should be equal but problems with the digital resolution might cause a small inequality. Next, change to 10, 90° pulses, and leave the relaxation delay at 0. This simulates ten pulses with a 4 sec interval between pulses. The slow-relaxing nucleus will be more saturated than the faster relaxer, and this should result in a spectrum with the slow-relaxing nucleus having a diminished intensity. To continue, repeat the run with a relaxation delay of 20 sec. This should produce a transformed spectrum with more nearly equal intensities, for the longer delay between pulses allows even the slow-relaxing nucleus almost enough time to relax. Try one more - this time going back to the 0 sec relaxation delay, but with 30° pulses. With the smaller flip angle, not as much time is needed for relaxation, so the signal intensities should be more nearly equal than they were when 90° pulses were used. Another suggestion, ask to see the individual frequencies in the FID. The frequency that is the more saturated will be seen to make a relatively small contribution to the total FID.

- 19) **Post-processing by a Lorentz-Gauss transform can help resolve peaks that are close together, even when they don't want to be resolved.** In our earlier discussion of resolving peaks, we concluded that the best approach was to collect data for long time periods. In order to do this we said that you needed long T_2 s, large transforms, and small spectrum widths. The Lorentz-Gauss transform is another way to enhance resolution. Before performing the FT, we multiply the FID by the expression $\exp(t/a-t^2/b)$. The values of a and b are chosen somewhat by trial and error. a is usually made to equal T_2 and b is made larger. To demonstrate this we should have a couple of closely spaced peaks, perhaps 20 and 20.5 Hz, of equal intensity, and don't worry about entering T_1 values. Other parameters to use are shown below:

Noise	0	Flip angle	90
T_2	1	Receiver gain	1
Acquisition delay	0	# of Pulses	1
Relaxation delay	0	Spectrum width	40
Quad detect		Points	512

When the FID is presented, click on <continue><no zero fill>. On the next menubar, click on <resolution enhancement> and enter 1 for a and 10 for b. You should see the envelope of your transformed FID superimposed on the original FID. Note that the envelope corresponding to the Lorentz-Gauss transform shows considerable intensity all the way from time=0 to time = 2-3 sec, and only then a tapering to nearly 0 at time=6 sec. At this point you may accept this transformation, or you may experiment with other combinations of a and b. Once you accept values for a and b you may continue to the Fourier transform and observe the results. Compare the resolution of several a,b combinations with that of the spectrum with no resolution enhancement.

Noise limits what you can do. You cannot amplify the latter stages of the FID, as we are doing here, without also amplifying any noise present. Try one with noise and see for yourself. One final comment about this operation: There is an upper limit to the extent of resolution enhancement. If you use a value of b that is too large, considerable distortion in the transformed spectrum will result. Try some.

- 20) **The amount of phase correction depends on the frequency!** The peak farthest from zero will need the largest correction. Fortunately, the correction is more or less linear as a function of frequency, so the software in your NMR data system should be able to do most of the work for you. To get a feel for how this happens, create three frequencies, perhaps -25, +30, and +35 Hz, with intensities of your choosing, leaving $T_1 = 0$. The remaining parameters are shown below:

Noise	.05	Flip angle	90		
T_2	1	Receiver gain	1		
Acquisition delay	.01	# of Pulses	1		
Relaxation delay	0	Spectrum width	64	Points	512

The transformed spectrum will show three peaks, all out of phase by differing amounts. (Just by accident one or two peaks might actually be in phase!!). When you ask for phasing, you will be told to mark a peak on the far left or far right side of the spectrum with the mouse. Once the peak is marked you will be asked to make the phase correction for that peak, as in Experiment 13. Next, the program assumes that a peak at a frequency of zero would need a zero phase correction, and any other peaks will be corrected by linear interpolation or extrapolation. Here is how it works: Suppose the peak marked needed 200° of correction. A peak at exactly half the frequency would get 100° of correction. A peak the same distance on the other side of zero would get -200° . There is only one problem. Sometimes the peak in the middle doesn't come into phase. This is because the peak on the left really needed 560° ($200+360$), or perhaps even ($200+360+360$). So if all of your peaks don't come into phase together, you try adding (or subtracting) 360° to the marked peak. The phase correcting routine recalculates the linear interpolation through $\nu = 0$, recalculates the expected phase correction across the entire spectrum, and then plots the spectrum with the new phasing. Once you get a spectrum that looks o.k., you quit. For your information the phase correction (in deg) is shown below the spectrum.

The phasing in an actual experiment need not extrapolate to zero at zero frequency, for there are instrumental factors that impose a constant phase error (a zero-order error) across the entire frequency range, as well as the linear error simulated here. We chose to include only "first-order" phasing in this simulation.

- 21) **Aliased peaks will not 'phase' properly.** Repeat the above run, but this time use a spectral width which is less than the highest frequency in your spectrum. This will give you aliasing, as described earlier. Now try to do the phase correction. You should usually find that the aliased peak causes trouble.