MARAN Ultra
Non-Expert
User Manual

MARAN Ultra Non Expert User Manual
v1.7 April 2006

Note: It is recommended that users access the CD-ROM supplied with the instrument for the latest version of this manual.

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Contents

Introduction

The MARAN Ultra Non Expert Users Manual provides information on the following:

1. How to construct a setup sample for use with MARAN instruments.
2. How to set up system parameters and acquire data using the FID pulse sequence.
3. How to acquire T_2 relaxation data using the CPMG pulse sequence.
4. How to acquire T_1 relaxation data using the INVREC pulse sequence.
5. How to conduct diffusion experiments using the DIFF pulse sequence
6. How to conduct 1D profile experiments using the PROFILE pulse sequence.
7. How to set the 90 and 180 degree pulses using TRAIN90 and TRAIN180
8. NMR hardware description.
9. Information on how to tune RF probes using WOBBLE.

The MARAN Ultra Non Expert Users Manual does not attempt to:

- Educate the user in the fundamentals of low resolution NMR. OIMBL recommends the following texts for learning basic Fourier transform NMR techniques:

  Farrar, T. C. and Becker E. D. (1971) *Pulse and Fourier Transform NMR*, Academic Press, New York.
  Callaghan, P. T. (1991) *Principles of Magnetic Resonance Microscopy*, Oxford University Press, (ISBN 0-19-853997-5).
  Hills, B (1998) *Magnetic Resonance Imaging in Food Science*, Wiley, (ISBN 0-471-17087-9).

- Educate the user in operating the Windows operating system. The manual assumes the user understands most of the basic Windows concepts including clicking, dragging, filenames, directory structures and menu operation. Users requiring a more detailed description of RINMR's functionality should refer to the RINMR User Manual.

Chapter 1 Setting System Parameters

1.0 Introduction

Setting up the MARAN instrument normally involves configuring several system critical parameters. Some of the parameters are time invariant, i.e. once set up correctly they do not need to be changed.

The system parameters are SF (spectrometer frequency), O1 (frequency offset), P90 (90º RF pulse length), P180 (180º RF pulse length) and Dead1 (RF probe dead time).

This chapter explains how to view and set the system parameters. Data acquisition using an FID pulse sequence is also described.

1.1 Preparing a setup sample

Before an NMR system can be set up correctly it is necessary to make a setup sample. This is a sample that is used to set up the instrument parameters.

It is a good idea if the setup sample is time stable (i.e. does not undergo any chemical transformation or degradation over time), as then it can be kept to test the instrument for GLP purposes.

Examples of good setup samples include:

- Glycerol
- Mineral oil
- Dodecane
- Doped water (with $CuSO_4$ to reduce the T_1)

Bad setup samples include:

- Any water sample (long T_1).

The sample tube should be filled (up to the first white line if in doubt) and sealed with a rubber bung and parafilm.

Knowing the total mass of the tube is useful in case any leaks are suspected.

1.2 Suggestions for GLP

It is suggested that once a setup sample is obtained an FID should be recorded and the parameters used to produce it saved to disk in a separate directory.

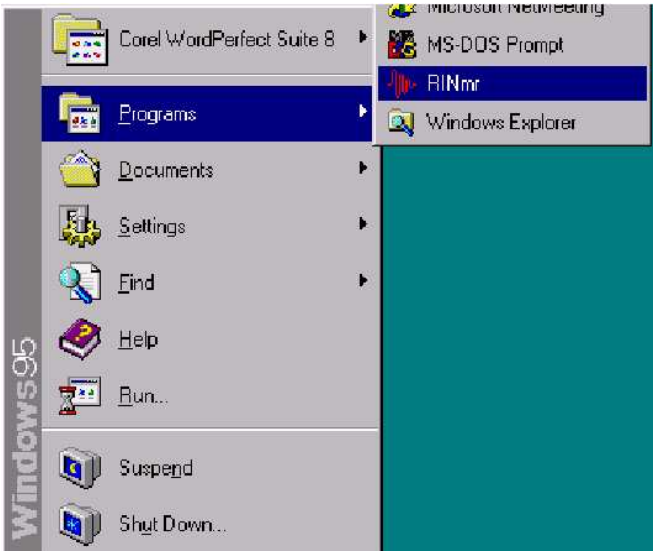
Once per month or depending on GLP requirements the setup sample should be re-measured and compared with the existing files. If significant deviations are observed then OIMBL should be contacted.

More information on how to perform this procedure may be found in the GLP manual.

1.3 Setting up the system parameters and acquiring data with the FID pulse sequence

Before NMR experiments can be performed the system parameters must be set up correctly. The system parameters are parameters used in NMR experiments that are system dependent, i.e. they vary from system to system. Once the system parameters are set they tend to stay constant, although some may vary slightly on a daily basis. Automatic setup routines allow users to set the most critical system parameters. When the RINMR software is installed a factory determined set of system parameters is installed. Section 2.1 gives a brief explanation of what the system parameters do and how to find out what they are. We recommend you record the factory values of the system parameters so that you have a permanent record for your future reference.

1.4 Viewing the system parameters



To view the system parameters, first place the setup sample in the magnet and run RINMR from the Windows Start Menu:

Place RINMR in acquisition mode by clicking on the acquisition radio button.

A pulse sequence is a series of instructions that tell the NMR instrument what kind of experiment to perform. To set up the instrument the simplest experiment should be used. This experiment is called an FID. Load in the FIDpulse sequence (FID.EXE).

You are now ready to view the basic system parameters, which are described in the following sections.

1.4.0 SF (Spectrometer Frequency)

SF controls the frequency of the radiofrequency pulse that is applied to the sample. The frequency of the applied radiofrequency pulse must match the energy gap between the two nuclear energy levels. The spectrometer frequency is determined by the following equation:

$$\nu=\gamma B/2\Pi$$

Where γ is the magnetogyric ratio of the hydrogen nucleus (often referred to as a proton or 1H), B is the magnetic field strength in Tesla (T) and ν is the spectrometer frequency.

Because ω and B are directly proportional, NMR scientists often state magnetic field strengths in terms of frequency units (Hz) rather than Tesla (T). For example, a 0.54 T magnet can be described as a 23 MHz magnet.

Typical MARAN Ultra magnet frequencies and their field strengths are shown below:

Magnet Field Strength (T)	Magnet Frequency (1H, MHz)
0.046	2
0.117	5
0.235	10
0.524	15
0.47	20
0.54	23

The SF required is dependent on the magnet type. The magnets are named after the approximate value of SF required. For example MARAN 23's require an SF value of approximately 23 MHz.

To obtain the current value of SF type SF in the RINMR command box and press return:

This is the factory installed value of SF (it will probably be different from the value shown above) and it should be close to the nominal frequency strength of the magnet (i.e. 23.0 MHz in the case of the MARAN 23). Write this value in the Appendix at the back of this manual.

The value for SF is invariant with time. Any variation in magnet frequency is accounted for by varying O1 (see below).

1.4.1 O1 (Offset 1)

Traditionally the frequency of the RF pulse is represented not by simply SF but by SF plus an offset. Over time the frequency of the magnet may drift slightly. This drift is accounted for by altering O1 rather than SF. Note that O1 is measured in Hz and SF is measured in MHz.

To obtain the current value of O1 type O1 in the RINMR command box. This is the factory installed value of O1. Write this value in the Appendix at the back of this manual.

O1 varies on a daily basis, so before performing a series of experiments O1 should be set correctly. Setting O1 can be time consuming, so an automatic setup script exists to allow users to set up O1. How to use the setup script is described in section 3.3.

1.4.2 P90 (90º pulse length)

Once the correct frequency of the RF pulse has been determined we need to establish how long the RF pulse should be applied to obtain the largest NMR signal. In an NMR experiment, the signal amplitude varies as:

$$NMRSignal \propto \sin(\theta)$$

where θ (known as the tip angle) is directly proportional to the length of the RF pulse.

When θ=90º (or $\pi/2$) a maximum is observed in the signal amplitude. This is known as the 90º pulse. If θ=180º (or π) a minimum is observed in the signal amplitude.

To set the value of θ to achieve a 90º pulse we perform many different NMR experiments, each with a different value of RF pulse length. As the RF pulse length increases, the signal amplitude obtained increases until we achieve a 90º pulse.

The length of the RF pulse on the MARAN Ultra spectrometer is controlled by the parameter P90.The value for P90 necessary to achieve a 90º pulse depends on the type of RF probe (10mm probes have smaller P90's than 26 mm probes) and the type of RF amplifier (25W amplifiers produce longer 90º pulses than 300W amplifiers). In addition, the construction of the probe affects the value of P90 that will achieve a 90º pulse. No two probes will have exactly the same 90º pulse length, even if they are of identical design and are used with identical RF amplifiers.

To obtain the current value of P90 type P90 in the RINMR command box. This is the factory installed value of P90. Write this value in the Appendix at the back of this manual.

P90 varies slightly depending on sample type. Like O1, there is an automatic setup script that allows users to set P90 automatically. How to use this is described in section 3.3.

1.4.3 Dead1 (Probe dead time)

After the RF pulse has been applied to the sample the probe 'rings'. During this time no useful NMR signal may be acquired. This time is known as the dead time of the probe and varies (in a similar way to P90) from probe to probe.

We do not wish to acquire the signal while the probe is ringing, so we typically wait for a few microseconds after the RF pulse has finished until we begin to acquire data. This wait period is called Dead1.

To obtain the current value of Dead1 type Dead1 in the RINMR command box. This is the factory installed value of O1. Write this value in the Appendix at the back of this manual. Dead1 is invariant with time.

1.4.4 Dead2 and FW (Receiver dead time and filter width)

Filters may be applied to smooth the NMR signal and reduce noise. The filter value is controlled by the parameter FW. MARAN Ultras usually have two filter settings, 1000000 Hz (or 1 MHz) or 100000 Hz (or 100 KHz).

To obtain the current value of FW type FW at the RINMR command line.

When a filter is applied to an NMR signal an extra wait period is needed before useful data may be acquired. During this time the filter stabilises. The wait period is called Dead2 and varies depending on the filter used.

Narrowband filters require long values of Dead2, wideband filters require short values. The relationship between FW and Dead2 is fixed and as follows:

MARAN 2 Instruments: FW=1000000Hz, DEAD2=5μs. FW=100000Hz, DEAD2=20μs.

Other MARAN Ultra Instruments: FW=1000000Hz, DEAD2=3μs. FW 100000, DEAD2=15μs.

WARNING: If in doubt set FW to 1000000Hz and Dead2 to 3μs (5μs on MARAN Ultra 2 MHz instruments). Do not change the filter settings unless you understand the consequences!

Chapter 2 FID Experiments

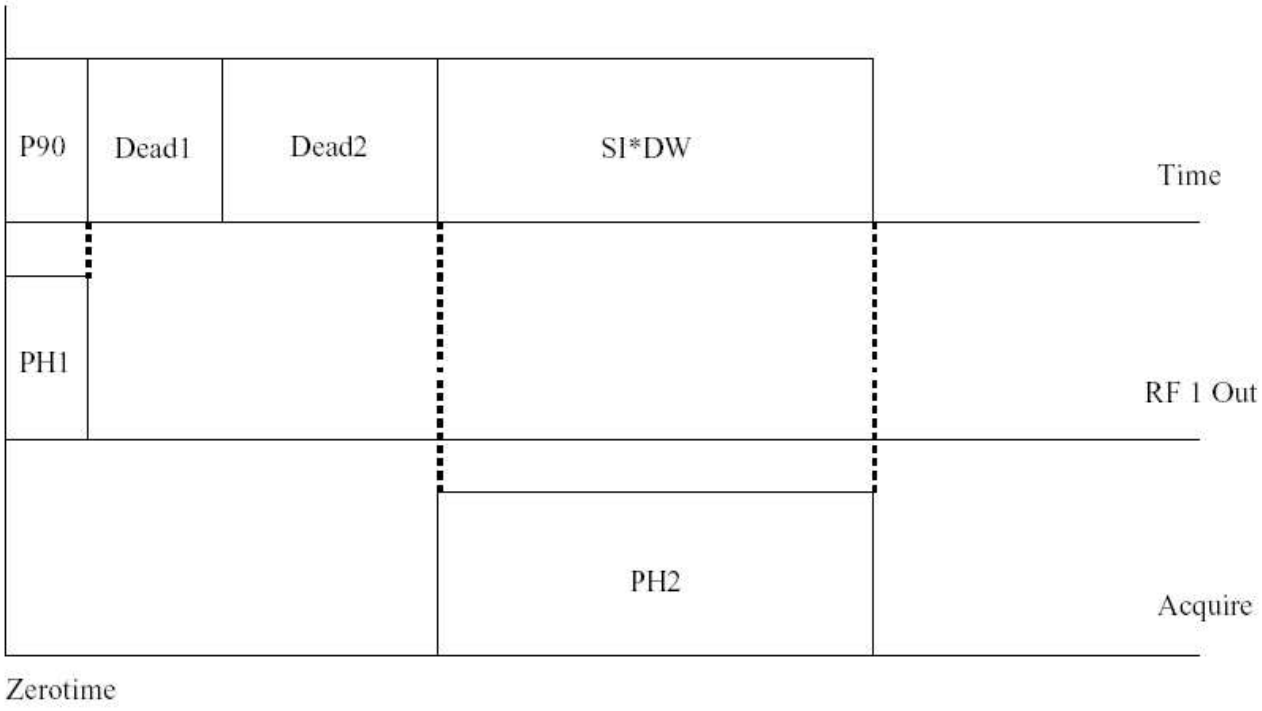
2.0 Introduction

The simplest NMR experiment is called an FID (Free Induction Decay) experiment (or application). A single RF pulse (normally a 90º pulse) is applied to the sample and the response is measured as a function of time after the RF excitation. Because of its simplicity, the FID application is often used to set up the NMR instrument.

The system parameters are system wide parameters that are used in all NMR applications. However each individual application has its own specific parameters which are specific to that particular application.

In the previous section we explained how to set up the system parameters. In this section we will set up the application parameters that will allow us to run an FID application.

2.1 The FID pulse sequence



The pulse sequence timing diagram for the FID pulse sequence is shown above. The x direction (across the page) represents time. The zerotime label indicates the start of the experiment. The FID pulse sequence consists of a single RF pulse of duration P90 microseconds. Following this there is a wait period dead1 where the probe rings and a second wait period for the filters to stabilise, dead2.

After the dead times data is acquired. SI data points are acquired DW microseconds apart. This produces an NMR data set spanning a time period of DW*SI microseconds. After the experiment the system pauses for RD microseconds and the entire process is repeated.

The following sections describe the application parameters for the FID sequence in a little more detail, as well as some other parameters that are not included in the above timing diagram.

Set the parameters to their recommended values by typing the parameter name, i.e. SI, followed by the number in the RINMR command box. Ensure that the pulse sequence is set to FID (the currently loaded pulse sequence is displayed on the RINMR task bar) and that RINMR is in acquisition mode.

2.1.0 Size of acquisition buffer in points
SI is the number of data points acquired following the pulse. For setup purposes we recommend SI is set to 1024 (1K) of data points. Set SI 1024 on the RINMR command line.

2.1.1 DW (dwell time)
DW is the time between successive data point acquisitions. The total acquisition time following the RF pulse is therefore equal to SI*DW. For setup purposes DW should be set to 1μs.

In conjunction with the above setting of SI, this means 1024 us of data will be acquired following the RF pulse (and the dead time).

2.1.2 NS (number of scans)
NS is the number of scans that are acquired. Each scan is a separate NMR experiment. Multiples of 4 scans must always be used. The greater the number of scans used, the higher the signal to noise ratio of the data produced from the experiment. We recommend for setup purposes NS should be set to 4.

2.1.3 PH1, PH2, RFA0 and DS (phase list 1, phase list 2, RF amplitude channel 0 and dummy scans)
Descriptions of PH1, PH2, RFA0 and DS lie beyond the scope of this document. It is sufficient to say that for FID experiments these parameters should be set as follows:

PH1 0213
PH2 0213
RFA0 100
DS 0

Note that the recommended values for PH1 and PH2 are written in the RINMR Pulse Sequence and Script Manual, as well as in the acquisition parameters table (this will be described in a later section).

2.1.4 RG (receiver gain)
RG stands for receiver gain. This is the amount of amplification that is applied to the NMR signal before it is collected. If too much amplification is applied the NMR signal will saturate the instrument receiver and a warning signal will appear on screen.

If too little amplification is applied the signal will only occupy a few bits of the receiver making the resolution of the acquired data questionable.

The value required for RG varies depending on the instrument and the sample. For example a sample with more nuclei will give more signal and thus require a different setting of RG.

For example, if 3 grams of vegetable oil requires an RG of 6 to give a signal amplitude of 30, 6 grams of vegetable oil will require an RG of approximately 3 to give a signal amplitude of 30.

Set RG to 0.5 % via the RINMR command line. This is a low starting value that will accommodate almost all samples and instruments. We will set RG more accurately in the next section.

2.1.5 RD (relaxation delay)
RD stands for Relaxation Delay and is the time between successive scans. Typically several scans (NS) are performed and the results added to increase the signal to noise ratio of the data.

After the RF pulse is applied and the data have been acquired wait for the system return to its equilibrium state before performing the next scan. If you do not wait long enough until starting the next scan, the system will not have returned completely to equilibrium, which results in a decrease in signal amplitude for the next scan.

In order to obtain a maximum in signal amplitude and ensure that the amplitude from each scan is identical RD should be long enough to allow the system to return to equilibrium.

Note that the value for RD depends on the samples T_1 (the samples spin lattice relaxation time). NMR experts often set RD to 5 x T_1. Samples such as water have long T_1's, oil samples have short T_1's.

For the purposes of this experiment we will assume we are using an oil sample and set RD to a starting value of 1000000 microseconds (this can be entered using the shorthand 1S), which is approximately 5 x T_1 of most oils.

Later we will learn how to measure T_1 more accurately and set RD more precisely.

2.2 Viewing the parameters

We have now set up the instrument to start an FID experiment.

We can see a summary of the current parameters by clicking on the A button on the RINMR toolbar.

The toolbar and 'A' button.

Description	ID	Value
---SYSTEM---		
90 Degree Pulse (us)	P90	Your Value
Probe Dead Time (us)	DEAD1	Your Value
Receiver Dead Time (us)	DEAD2	3.0
Spectrometer Frequency (MHz)	SF	Your Value
Offset from SF (Hz)	O1	Your Value
---APPLICATION---		
Filter Width (Hz)	FW	1000000.0
Dwell Time (us)	DW	1.0
Size of Acquisition Buffer (points)	SI	1024
Number of Scans	NS	4
Receiver Gain (%)	RG	0.5
Relaxation Delay (us)	RD	1000000.0
90 Degree Pulse Phase List (rec: 0213)	PH1	0213
Receiver Phase List (rec: 0213)	PH2	0213
Dummy Scans	DS	0
RF Amplitude (%)	RFA0	100.0

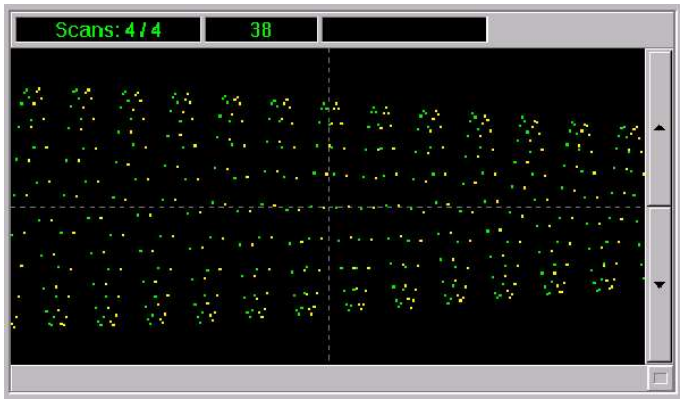
Check that your parameters match the values provided in the table above. Note that P90, SF, O1 and DEAD1 are instrument dependent and should be left at their current values.

2.3 Starting the FID experiment

To start the NMR experiment place the setup sample in the magnet. Make sure the instrument is in ACQUISITION MODE (click on the button to the right of the command line input).

Next type GS on the RINMR command line.

GS stands for Go Scans. The instrument will perform 4 scans, adding the scans together. At the end of the four scans the data is cleared and the process repeats. At the end of 4 scans the screen probably looks something like this:



Note the signal size in the middle box (in the above example signal size is 38). If the signal does not appear as above, try clicking on the up and down arrows to the right of the data set to magnify/reduce the display scale.

If the message CLIPPED is displayed in the end box:

reduce RG in 0.1 increments until it disappears by typing RG 0.4 (for example) on the command line.

Note that parameters may be changed while the experiment is running in GS mode. The parameters are updated after every NS scan.

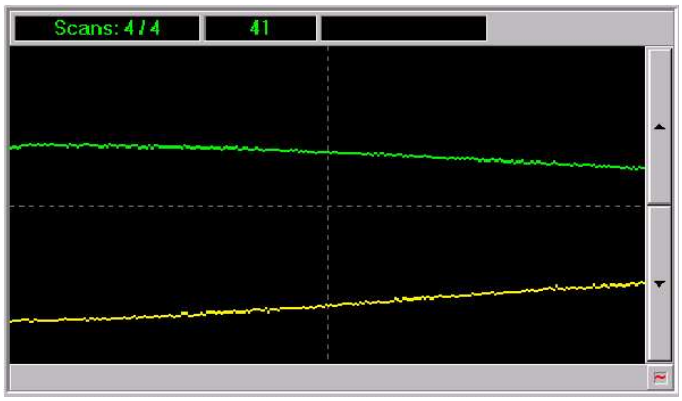
To stop the experiment press <CTRL> and <K> simultaneously.

The first thing to do is set O1 so the system is on resonance. To do this type .AUTOO1 on the command line (remember the '.' and the fact there is no zero in the syntax of .AUTOO1), or type RUN AUTOO1.

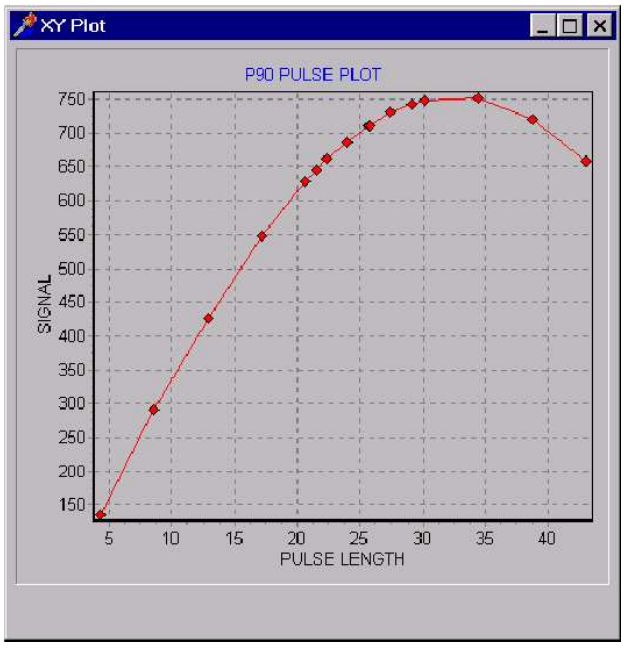
The instrument will now set up O1 automatically. At the end of the automatic setup it will report a value for O1. Click on the OK box to accept the value.

Type GS again.

You should now see that the oscillations in the two data channels have disappeared, and now two smooth decays take place, like the following:



The rate of the decay and the shape depend on both the sample and the magnet, so your decay will look slightly different to this.



Next P90 should be set up. Leave GS mode by pressing <CTRL>- <K> simultaneously. Type .AUTOP90 at the command prompt. As the automatic P90 setup runs you should see a graph like the one on the left.

As the value for P90 is increased, so the signal strength increases. Once the signal starts to decrease (i.e. the value for P90 is past the 90º pulse length) the experiment is repeated with smaller increases in P90. Finally, P90 and P180 (twice P90) are reported on screen (in the above example P90 is about 32us). The values obtained should be close to the values you wrote down earlier in the Appendix.

Finally set RG by typing .AUTORG at the command prompt.

The final parameter to set is RD and this must be set manually. Type GS to start acquiring data. Note the value of the signal size.

Decrease RD (note that the new value of RD will not be used until the next series of NS scans) in 100 ms increments and watch the value of the signal change. As you lower RD to 100ms the acquisition time for NS scans should decrease significantly (from 4 seconds to 0.4 seconds).

The size of the FID signal should also decrease in amplitude. This means that you are not waiting enough time for the system to return completely to equilibrium before remeasuring it.

Increase RD in 100ms increments until the signal size stops increasing (or only increases by, say, 1). At this point you are waiting long enough for the system to return to equilibrium.

At this point it is worth removing the oil sample and replacing it with a tube filled with a similar quantity of water (you do not have to stop the instrument running while you do this). Note down the RD for the oil sample, then try increasing RD until the signal size stops increasing for the water sample (note you may have to change RG is the CLIPPED message appears). Note how much longer RD is required for water than for oil. This is why we use oil as a setup sample, as it means we can acquire scans quickly with short values of RD and see the effect of changing parameters almost instantaneously, rather than the long period we have to wait for the water sample.

Note that if you use more water than oil you may have to reduce RG, otherwise the CLIPPED message will appear.

Finally, replace the water sample with oil and return RG (if changed) and RD to the oil values. After RD has been set it is wise to repeat the automatic setup routines again.

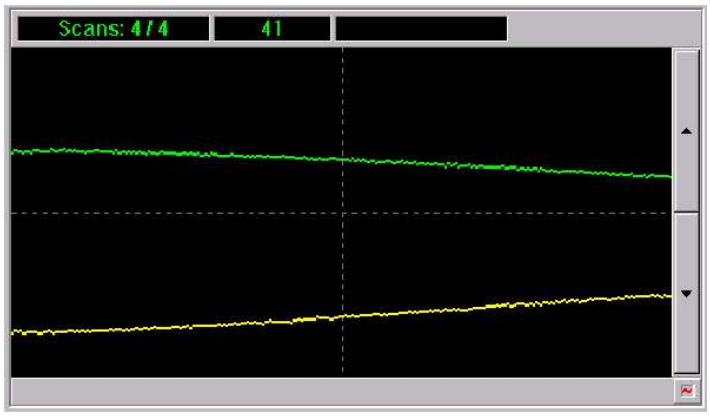
Type .AUTOO1 followed by .AUTOP90 followed by .AUTORG to repeat the setup process.

2.4 Other parameters

Next we will investigate the effect of changing some of the other parameters such as NS, SI and DW.

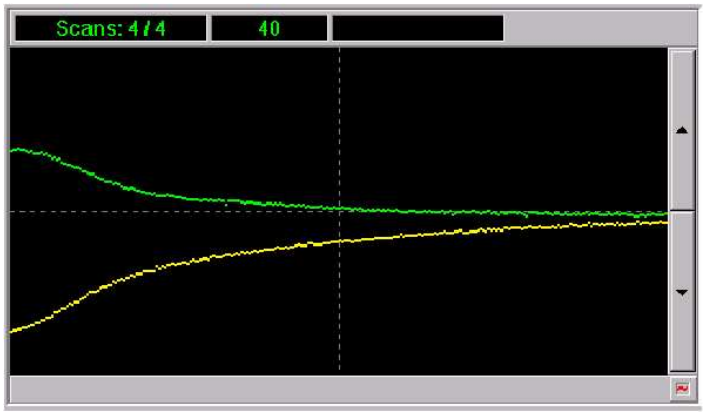
Set NS to 16, then type GS. The effect should be fairly obvious. The instrument now performs 16 scans rather than the 4 set earlier. Press <CTRL>-<K> to stop the instrument and return NS to 4. The signal to noise ratio of the data increases by a factor of 2 every time the number of scans is quadrupled (16 scans has twice the signal to noise ratio of 4 scans), but of course the time to perform the experiment increases by a factor of four. Remember that NS must always be set to a multiple of 4.

Press <CTRL>-<K> to stop the experiment. Set SI to 2048, then type GS. Note how the signal is acquired for a longer time. Increase SI until the signal decays into baseline noise - note that you cannot alter the value of SI while in GS mode - you have to stop the acquisition first. SI is the only parameter you cannot alter interactively in GS mode.



SI=1024 Points

DW was set to 1µs.



SI=8192 Points

The first 1/8 of the data set corresponds to the data shown above.

DW was set to 1µs.

Either DW should be increased or SI increased to acquire data into the baseline.

Finally note the effect of changing DW while in the interactive setup. Because the total acquire time is equal to SI*DW, changing DW by a factor of two has the same effect as changing SI by a factor of two.

However if DW is changed by a factor of two the curve shape will be less well defined. A DW of 1-5μs is usually a good choice. That way data can always be acquired into the baseline and be well characterised with a reasonable number of points (<8K).

Note that there is no point acquiring too much baseline noise - there is no information content in the noise and it may also affect data fitting and have to be removed at a later stage. Equally if you do not acquire down to the baseline you are effectively throwing away information that may turn out to be useful in the future.

2.5 Acquiring NMR data

Now you have learnt to use the interactive setup mode you need to learn how to acquire data for processing. To do this type GO at the command prompt.

The instrument will perform NS scans and then stop. The acquired data can now be seen on screen.

Note RINMR has now switched to PROCESS mode, ready to save the data to disk.

To save the data type WR on the command line. If you wish you can export the data to an Excel spreadsheet by typing EX.

Note to alter parameters you need to re-enter PROCESS mode.

2.6 Hints and tips on running FID applications

2.6.0 Setting RD

Setting RD is of critical importance, especially for samples with low signal to noise ratios. These samples require large amounts of scans (NS) in order to produce reasonable data. You may be tempted from the above experiments to set RD excessively long. This may lead to prohibitively long experiment times - or low(er) quality results.

2.6.1 The P90 setup script

The P90 setup script uses the current value of P90 as a 'seed' value for the P90 determination. Thus if P90 is set to 2 us and the actual 90º pulse of the instrument is 50 us the P90 determination will not function correctly. Always check that P90 is set close to the actual length of the 90º pulse (+/- 10 %) before running the .AUTOP90.

2.6.2 Solid samples

Solid samples have fast T_2's (the NMR signal decays very rapidly). If DW is set large, you may only acquire a few points at the beginning of the FID - i.e. the FID will not be well characterised. For solid samples it is usual to set DW as short as possible (0.1 us).

2.6.3 Setting NS

Setting NS to 4 cancels receiver artefacts in the NMR data. Failure to set NS to a multiple of 4 may lead to spurious results.

2.7 Running sample sets (experimental design)

Often you will wish to run several samples and compare the NMR data between samples. For the comparison to be valid the following points must be observed. You should note that these points are not only pertinent for the FID experiments, but also for CPMG and T_1 experiments).

2.7.0 P90

P90 MUST be kept constant (small variations in P90 will affect the absolute amplitude, but not the shape of the FID data). P90 does not usually vary from sample to sample unless the samples have significantly different conductivities or paramagnetic impurities. We recommend you set P90 at the start of your series of experiments and keep it constant throughout the series.

2.7.1 RD
Changing RD will affect the amplitude of the NMR signal. If you have a series of samples which require different RD's, you must set RD to the longest required value for the sample set.

2.7.2 O1
O1 varies on a daily basis. Although it is not necessary to set O1 before acquiring each individual data set, it is worth setting O1 before a series of samples are measured. Small variations in O1 will not affect the quality of the results if the system is put back on resonance. If the RF field is not set on resonance the quality of the pulse will be affected, for example a 'good' 180º pulse will no longer be achievable.

2.7.3 SI
To keep the screen display optimised SI should be set to a power of 2. This can be done by typing 1K, 2K, 4K etc.

2.7.4 RG
In order to compare data sets RG must be kept constant. If you are measuring a series of samples this means you must set RG so the receiver does not clip on the sample in the series that gives the largest signal - usually the sample with the largest mass - but not always, especially if the T_2 of the sample is short.

Note that it is extremely irritating to measure 50 samples and find that sample 49 clips the receiver! It is wise to set RG conservatively, so that the signal amplitude only occupies perhaps 70% of the receiver. This way you can be sure that no unpleasant surprises will occur. Note that many things can affect the amplitude of the NMR signal, including sample temperature.

2.7.5 Sample temperature
Sample temperature may have a dramatic effect on the shape and amplitude of the FID signal. In order to make valid comparisons between samples it is important to ensure that all samples in a series have identical temperatures.

This is particularly important as the MARAN Ultra operates at 35ºC rather than room temperature. A common mistake is to set up the NMR parameters on one of the samples (which may take 5-10 minutes). During this period the sample is changing temperature (eventually it will reach 35ºC). After the setup period, the setup samples temperature may be significantly different to the other samples in the sample set and it must be left to re-equilibrate before it is measured.

2.7.6 Summary
NMR is a uniquely powerful spectroscopic technique. Much of this power is due to the fact that the NMR signal is highly sensitive to a wide range of molecular characteristics. This sometimes means it can be difficult to isolate the particular characteristic we are interested in.

In order to make valid comparisons between samples observe the following rules:

Do not change the NMR parameters between experiments on individual samples in the series (the exception is O1).

Ensure that samples have identical temperatures before they are measured.

These precautions help ensure that any differences between samples we observe are due to changes in sample properties and not due to experimental protocol. It should be noted that these procedures are good scientific practice for any type of measurement and apply to all experiments, not only to NMR experiments and FID's.

2.8 Notes on analysing FID data
NMR data is acquired in quadarature, i.e. two channels of data are acquired simultaneously. The relative amounts of data in each channel is called the phase of the data.

When data is acquired it is usually displayed in quadrature mode. This allows us to see easily whether the system is off resonance.

In low resolution NMR, the phase of the data is almost always instrument dependent and provides no useful information about the system. It is usual to take the complex magnitude of the acquired data before data analysis. This may be done by typing MAG on the RINMR command line after the data have been acquired and produces a single channel which can be fitted to an exponential.

The RINMR display can be changed to magnitude mode by selecting View-Magnitude from the menus. Note that you will still have to type 'MAG' to take the magnitude of the data after it has been acquired.

Chapter 3 CPMG Experiments

3.0 Introduction

Although FID experiments are useful for setting up NMR instruments, they have some disadvantages.

The main disadvantage is that the decay constant (or shape) of data acquired with FID's is affected not only by the sample properties, but also the homogeneity (or uniformity) of the magnetic field over the sample.

Most scientists are only interested influence of sample properties on the NMR data, so removing the effect of the magnetic field on the decay of the NMR data would be extremely useful.

Fortunately the effects of the magnet on the signal can be removed by using a special pulse sequence called a CPMG experiment. CPMG stands for Carr Purcell Meiboom Gill (it is named after the people who invented it). A timing diagram for the CPMG pulse sequence may be found in the RINMR Pulse Sequence manual.

The CPMG sequence involves applying not a single RF pulse to the sample, but many pulses. Each time a pulse is applied the signal decay to the magnetic field is removed and a single data point is acquired. The decay of the data therefore only reflects the sample properties and for liquids is exponential in nature (a single exponential for water and a distribution for oil). The decay constant of the exponential is called T_2 or the spin-spin relaxation time.

3.1 Running a CPMG experiment

Load in the CPMG pulse sequence (in the same manner as you loaded the FID pulse sequence earlier, remember to load in a pulse sequence RINMR must be in ACQUISITION mode). Note that there are several types of CPMG pulse sequence (CPMG, CPMGF, CPMGGRAD etc, be sure to select the correct one - CPMG). The CPMG Pulse sequence has different parameters than the FID pulse sequence. Click on the 'A' button to view the parameter set:

Description	ID	Value
---SYSTEM---		
90 Degree Pulse (us)	P90	Your Value
180 Degree Pulse (us)	P180	Your Value
Probe Dead Time (us)	DEAD1	Your Value
Receiver Dead Time (us)	DEAD2	3.0
Spectrometer Frequency (MHz)	SF	Your Value
Offset from SF (Hz)	O1	Your Value
---APPLICATION---		
Filter Width (Hz)	FW	1000000.0
Dwell Time (us)	DW	1
Points per Echo (points)	SI	1
Number of Echoes	NECH	256
Number of Scans	NS	4
Receiver Gain (%)	RG	1.00
Relaxation Delay (us)	RD	1000000.0
90-180 Degree Pulse Gap (us)	TAU	1000
90 Degree Pulse Phase List (rec: 0213)	PH1	0213
Receiver Phase List (rec: 0213)	PH2	0213
180 Degree Pulse Phase List (rec: 1122)	PH3	1122
Dummy Scans	DS	0
RF Amplitude (%)	RFA0	100.0

The extra parameters are:

3.1.0 P180 (180º pulse length)
These are the rephasing pulses that are applied to remove the effects of the magnetic field on the NMR data. Each rephasing pulse is approximately twice the 90º pulse (P90) in length (therefore it is termed a 180º pulse). A certain time after each P180 pulse has been applied an 'echo' occurs in the signal. SI points are sampled at the top of the echo DW microseconds apart. Most CPMG data sets are acquired with a single point for each echo (SI=1) so DW has no meaning.

3.1.1 NEch (number of echoes)
The number of echoes that will occur (equal to the number of rephasing pulses applied). If SI is set to 1, NEch data points will be acquired. NEch in the CPMG sequence may be thought of as the equivalent of SI in the FID sequence.

3.1.2 Tau
This is the time between successive rephasing pulses. A gap of 2 x Tau is left between each pair of rephrasing pulses. If SI is set to 1, 2 x Tau in the CPMG sequence may be thought of as the equivalent of DW in the FID sequence.

3.1.3 PH3 (phase program 3)
This is the phase list for the rephasing pulses. Note this should be set to 1122 as indicated. Failure to set this parameter to 1122 will result is spurious data being acquired.

3.2 Acquiring data using CPMG
Data may be acquired in the same way as before using the FID pulse sequence. Using a tube of oil optimise NEch and tau in GS mode such that the signal decays into the baseline. Note as changing NEch changes the size of the acquisition buffer it may not be changed interactively.

Once you have optimised the parameters acquire the data using GO. Once the data have been acquired type T_2 to fit to a single exponential.

Repeat the experiment with a water sample (remember to change RD and RG if necessary!).

Try T_2 again and find out the time constant of the decay.

3.3 Hints and tips on acquiring and analysing CPMG data

3.3.0 Setting tau value
Although the analogy of the CPMG tau parameter with the use of DW in the FID pulse sequence is useful, it should not be assumed that varying tau simply extends the timescale of the CPMG sequence.

Changing tau will often change the shape of the CPMG decay. This is because of molecular diffusion and exchange processes that may be taking place within the sample. Users should examine the text by Hills for more information on how changing tau may affect the relaxation decay.

To see individual exchange processes and minimise diffusion contrast, tau should be set as short as possible. To see the average of molecular exchange processes and maximise diffusion contrast tau should be set as long as possible. Note that diffusion contrast may be increased by the use of magnetic field gradients.

3.3.1 Baseline offsets due to magnitude calculations

Often in low resolution NMR, data are fitted in magnitude format, i.e. the complex magnitude of the two quadrature channels is taken to produce the final signal. Normally, the NMR data is not usually phased - i.e. all the signal does not reside in a single channel.

The result of taking magnitude data can lead to errors in data fitting - especially when using low signal to noise data (such as that obtained from rock cores). Specifically taking magnitude data leads to all noise in the data becoming positive, which gives the data a baseline offset. In high signal to noise data sets the offset is often insignificant. However in low signal to noise data the baseline offset due to noise resulting from the magnitude calculation may become significant. The baseline offset is indistinguishable from long time constants present in the data, and may lead to spurious results in the data fitting.

It is therefore usual to phase rotate NMR data prior to exponential (distributed and discrete) fitting. This is a mathematical process which transforms the data so it resides in a single channel. Data phasing may be performed using the RINMR data acquisition software using the ROT command - further instructions may be found in the RINMR user manual.

3.3.2 Vibration

As CPMG experiments are sensitive to diffusion, any extra motion will result in distortion of the data. Users should take care to ensure that no vibration of the instrument or sample movement occurs during the CPMG experiment.

Chapter 4 T_1 Experiments using INVREC

4.0 Introduction

The return of the magnetisation to equilibrium following its pertubation by an RF pulse is governed by T_1, also known as the spin lattice relaxation time.

This section describes how to make T_1 measurements using the inversion recovery method.

4.1 Initialising parameters

First of all the system and application parameters should be set up using the FID pulse sequence. Once this has been performed the INVREC pulse sequence should be loaded. Set the INVREC application parameters to the same parameters as those used in the FID pulse sequence. Ensure that SI is set to 8.

In addition to the parameters found in the FID pulse sequence the following parameters exist:

D1

This is the time period between the inversion pulse and the read pulse

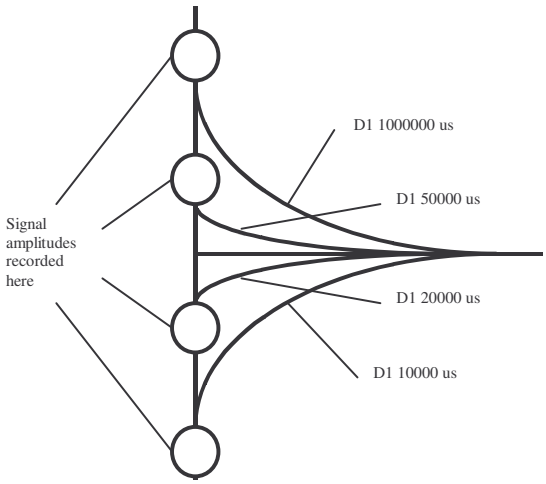
It should also be noted that the recommended values for PH1, PH2 and PH3 are different for inversion recovery experiments. They should be set to:

PH1 1
PH2 0213
PH3 0213

Check your parameters by clicking on the 'A' button on the toolbar.

4.2 Making a T_1 measurement

In order to measure T_1 many experiments must be performed, each with a different value of D1. The amplitude of the signal acquired for each different value of D1 is recorded and plotted against D1, producing an exponential curve which has a decay constant T_1:



A script has been written in order to facilitate the acquisition of T_1 data. The script is called T_1. To run the T_1 script type .T_1 at the RINMR command prompt.

The T_1 scripts prompts for two filenames, the first is the name of a list which contains the different T_1 values. Either select an existing list or enter your own.

For a mineral oil sample the following list should suffice:

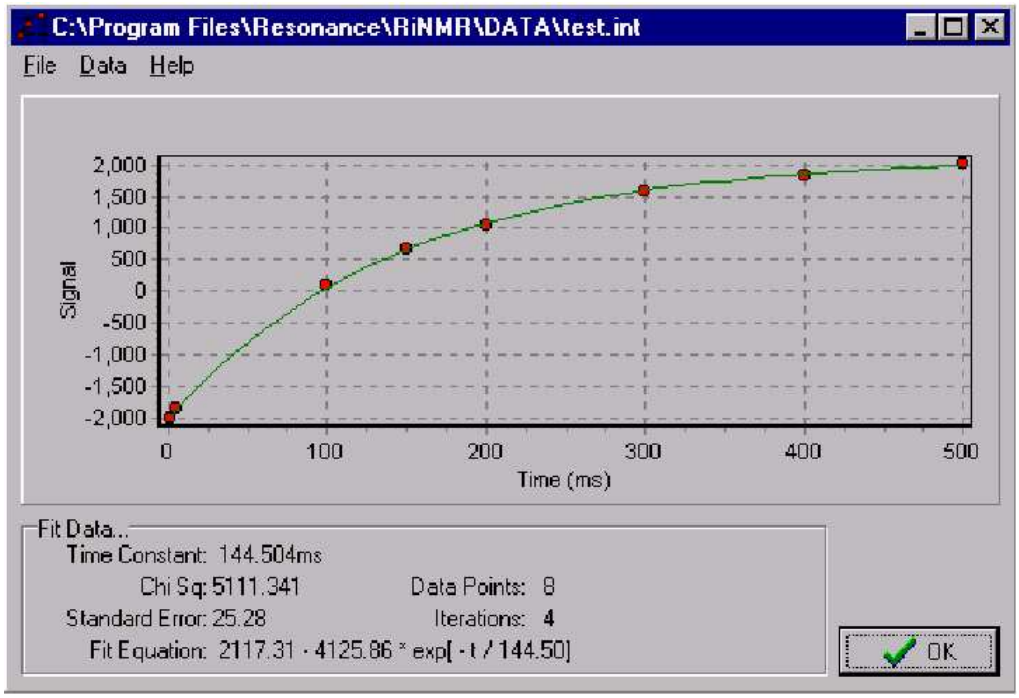
1000
5000
10000
20000
50000
100000
200000
400000
1000000

After the list has been entered, the script prompts for a file prefix to save the data to. Type a filename (for example moil). The data will be saved to the data files moil.00001.Ridat, moil.00002.ridat etc.

Once the filename has been entered the script will acquire the T_1 data.

When the script finishes the data is automatically phase rotated (a description of why phase rotation is necessary is beyond the scope of this document) and the amplitude of the data acquired for each D1 value is determined.

The script prompts for a filename to save the T_1 data to (an .int file, these can be used at a later stage with WinFit and WinDXP if necessary) and then automatically fits the data to a single exponential.



The T_1 value can be found in the data display below the graph.

4.3 Notes on Performing T_1 Measurements

4.3.0 Establishing D1 values

D1 values should vary from near zero until all the magnetisation has returned to equilibrium (this value is sometimes known as tau infinity). As a rough guide 5 x T_1 allows 95% of the magnetisation to return to equilibrium.

4.3.1 Choosing RD

It is recommended that RD is set to tau infinity.

4.3.2 Choosing SI

Only the first 8 points of each data set are used in the T_1 calculation. Thus acquiring more data sets with SI>8 will not serve any useful purpose.

Chapter 5 Diffusion Experiments using DIFF

5.0 Introduction

Diffusion constant measurements may be made using the DIFF and DIFFA pulse sequences. In a similar manner to T_1 measurements, diffusion measurements require several separate experiments to be performed in order to calculate the value of the diffusion co-efficient.

5.1 Magnetic field gradients

Diffusion constant measurements require the use of magnetic field gradients. In order to conduct gradient experiments two pieces of equipment are required; a gradient probe and a gradient amplifier. The standard supplied gradient amplifier is a Crown Macrotech.

To turn on the Crown amplifier press the on/off switch on the left of the front panel. Two green status lights should illuminate, after several seconds these will switch off and the two red lights above will illuminate. This indicates that the gradient amplifier is functioning correctly. If the amplifier does not do this a fault is present on the system.

WARNING: Users should refer to MARAN Ultra supplementary equipment manual and the manuals supplied by Crown for additional information on the gradient amplifiers.

5.2 Testing the gradient amplifiers using the degauss script

When the magnetic field gradients are used they disrupt the static magnetic field produced by the permanent magnet in the MARAN Ultra spectrometer. This results in a decrease in the homogeneity of the permanent magnet.

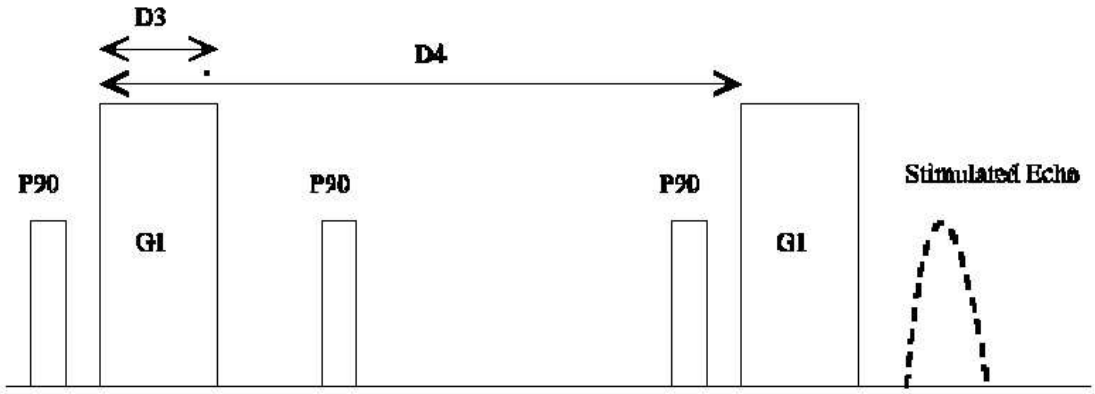
To restore the magnet homogeneity the Degauss script can be used. The Degauss script may be executed by typing '.Degauss' in the RINMR command box.

When the Degauss script is executed there is a short wait of approximately 4 seconds followed by a noise that sounds like a gong being hit. The volume of the noise depends on the system configuration and it is often quite faint.

If the noise does not occur when the amplifier is switched on and the Degauss script is executed then there is a fault with the gradient system. Please contact OIMBL for advice.

Finally it is recommended that the Degauss script is run before gradient scripts are executed (to place the magnet in a known state) and after gradient experiments are executed (to ensure that the magnet is left in a known state).

5.3 Diffusion measurements



Diffusion measurements are made with the DIFF and DIFFA pulse sequences. A simplified timing diagram for the DIFF pulse sequence is shown above. The DIFF pulse sequence consists of three 90º pulses and two magnetic field gradient pulses. The first 90º pulse tips the magnetisation into the transverse plane (like an FID experiment). While the magnetization resides in the transverse plane it is subjected to a gradient pulse. The amplitude of this pulse is known as g, the duration of the pulse is often labelled δ. This pulse labels the positions of the spins. After the pulse has been applied the spins are rotated back along the z axis by the second 90º pulse (note the phase change). A long period, often know as Δ is then left for the spins to diffuse. After waiting for Δ a second gradient pulse of amplitude g, duration δ is applied to read the positions of the spins. An echo known as a stimulated echo (similar to a spin echo) is created. The amplitude of the echo formed is dependent on:

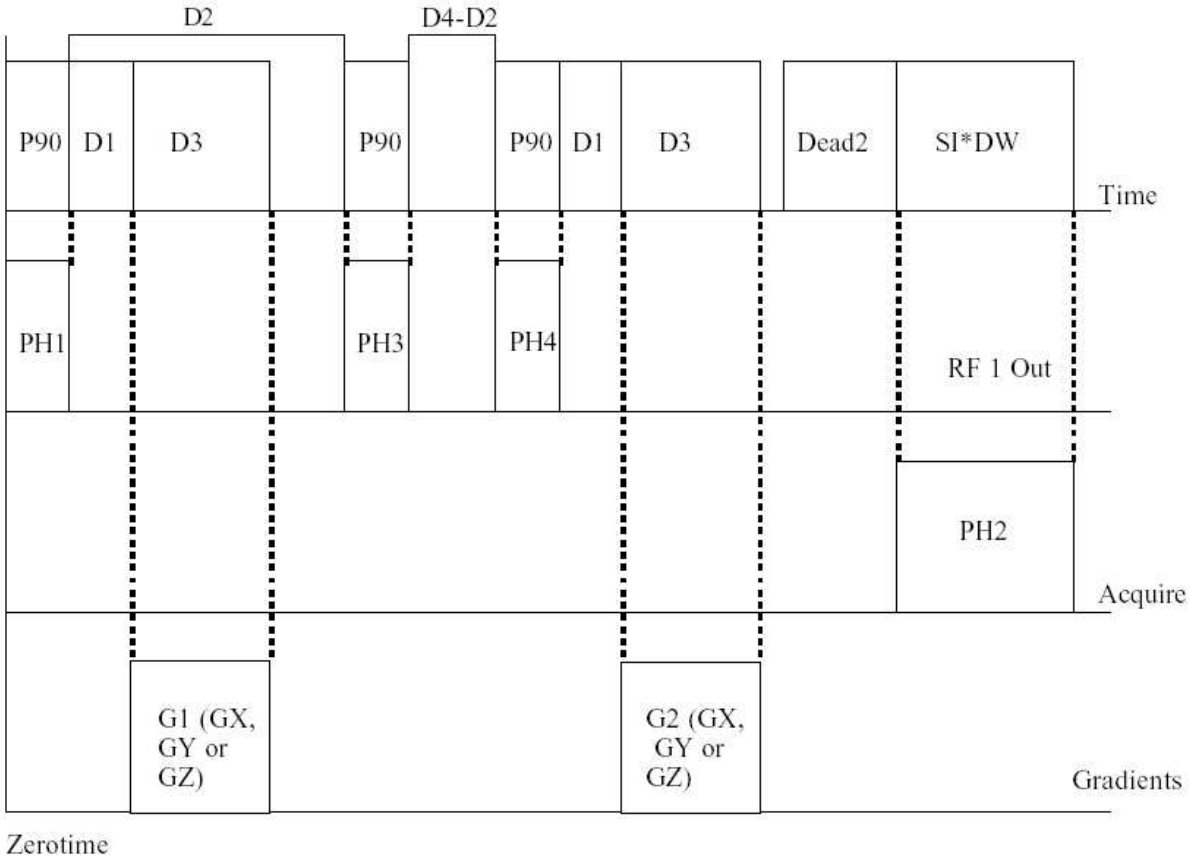
1. How far the spins have moved between the two gradient pulses (i.e. the diffusion co-efficient of the molecules).
2. How far the gradient pulses are apart (often referred to as Δ).
3. The duration of the gradient pulses (referred to as δ).
4. The strength or amplitude of the gradient pulses (referred to as g).

To calculate the diffusion co-efficient several experiments are performed with different combinations of δ, Δor g. The individual experiments are often referred to as Q-steps. The following graph is then plotted:

Log (Echo. Amp) vs. $(\gamma * g)2 * \delta 2 * (\Delta - \delta/3)$

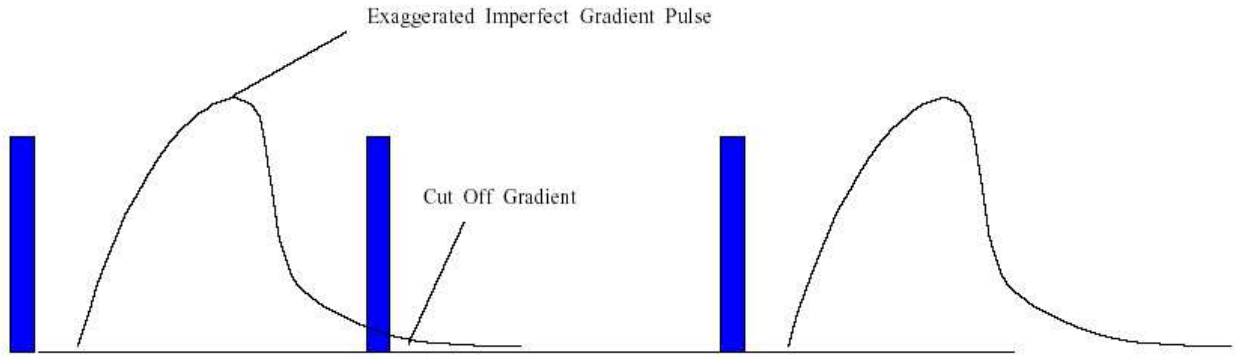
where γ is 267538030 $s^{-1}T^{-1}$. The diffusion coefficient is the gradient of the best straight line fit to this graph. Note g should be in T and δ and Δ in seconds.

Below is a diagram of the pulse sequence DIFF supplied with RINMR:



The RF event line (top) shows the three 90º RF pulses and the area where the echo is acquired (SI*DW). The gradient event line (bottom) shows the two gradient pulses.

After the first 90º pulse there is a short wait period D1 before the first gradient pulse is applied. This is usually set to 100 microseconds. The first gradient pulse follows this, and has duration D3 (δ) and amplitude G1 (g). D2 is the gap between the first two 90º pulses, so after the gradient pulse there is a gap of approximately D2-D3-D1 microseconds before the second 90º pulse is applied. The time between the end of the first gradient pulse and the second 90º pulse is quite critical. The diagram below indicates why:



In reality the gradient pulses are not square but have finite rise times and fall times. The fall time is dominated by eddy currents. If the duration between the end of the first gradient pulse and the second 90º pulse is not sufficient, part of the gradient pulse is cut off by the second 90º pulse.

It is essential that D2 is large enough compared with D3+D1 to allow the gradient pulse to fall to zero before the second 90º pulse is applied. The value of D2 necessary is dependent on:

- The value of G1 (g) used.
- The type of gradient set.
- The type of magnet.
- The type of gradient amplifier.

The value of D2 also varies between identical specification systems due to design tolerances, so it is difficult to provide an exact formula for calculating what D2 should be. As a rough guide, D2 should be at least 2ms longer than D3+D1 at the very minimum and if D2 is not set long enough it almost always results in the plot of equation [1] being non linear.

Users may be tempted from the above information to set D2 to an excessively long value to prevent corruption of the first gradient pulse by the second 90º pulse. However it should be noted that the longer the value of D2, the lower the signal to noise ratio will be of the final echo. This is because while the magnetisation resides in the transverse plane it is subject to T_2* relaxation (magnet inhomogeneity). There is much to be gained by making D2 as short as possible.

D4 (Δ) is the time between the two gradient pulses. Although during the period D4 the magnetization lies along the z axis (and thus is unaffected by T_2/T_2* relaxation) it is still subject to T_1 relaxation. Thus making D4 too long may lead to a reduction in signal to noise ratio of the echo.

G1 (g) is the gradient strength of both the gradient pulses. In general it is desirable to keep G1 as low as possible in a diffusion experiment - the lower the value of G1 the more chance the gradient pulse has of being square - the time to reach maximum amplitude (the rise time) will be shorter, and the fall time will be shorter, allowing decreases in D2. However, if G1 is too small, D4 and D3 (and consequently D2) may have to be much larger to achieve significant echo attenuation.

SI*DW is the acquisition time across the top of the echo. Ideally, as much of the flat top of the echo as possible should be sampled and averaged to achieve the best signal to noise ratio. Often on liquids a filter setting of 100 KHz is appropriate. Note that if more than the flat top of the echo is sampled and used in the diffusion calculation the echo attenuation values will acquire a T_2* weighting.

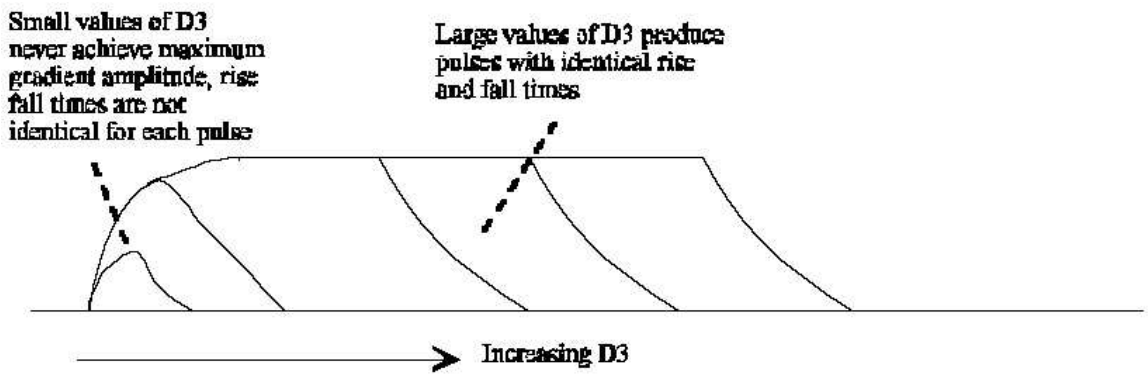
5.4 Varying D3 (δ), D4 (Δ) and G1 (g)

We have seen that in order to perform a diffusion experiment we must perform several q steps. This can be done by performing several different experiments varying either δ, Δ, g or indeed all three variables.

The aim is to perform measurements at different values such that the echo is *just* attenuated into the baseline noise of the spectrometer. This gives the maximum range of attenuation and the best measure of the diffusion constant. The pros and cons of altering the three different variables will now be discussed.

5.4.0 Varying D3 (δ)

The calculation of the diffusion co-efficient relies on perfect rectangular gradient pulses. We have seen that in practicality this is not possible due to the finite rise and fall times of the gradient pulses. The diagram below shows the effect of varying D3 on imperfect gradient pulses.



It can be seen that because of the finite rise times, the first few values of D3 are significantly less ideal than the final few. This is because the rise/fall times constitute a larger proportion of the total gradient pulse duration.

Varying D3 does have the advantage, however, that the value of D2 required to ensure that the first gradient pulse is not corrupted by the second 90º pulse will be similar for each value of D3, as once the gradient strength has achieved maximum the fall time will be the same for each value of D3.

The above diagram also shows that it is unwise to choose small values of D3 (especially if G1 is large). The larger G1 is, the longer it takes for the gradient to reach a maximum value and the longer D3 must be to achieve a reasonable shaped gradient pulse. This of course competes directly with the requirement to keep D2 small. Too small values of D3 show themselves as non linearities in equation [1] (small values of D3 do not produce as much attenuation as they should, compared to large values of D3).

5.4.1 Varying D4 (Δ)

At first glance varying D4 appears to be the most favourable way of increasing the effective magnitude of each Q step. However varying D4 not only subjects the echo to attenuation due to the increase in effective diffusion time, but also attenuation due to T_1 relaxation. Because of the relationship in equation [1], relatively large changes in D4 (compared with D3 and G1 which appear as squared terms) are required to increase the magnitude of each Q step significantly. Varying D4 can only be valid if the T_1 of the sample is very long compared to the variations in D4.

Varying D4 does have the advantage that the gradient rise times and cut-offs will be identical for each Q step, as G1 and D3 will not change.

5.4.2 Varying G1 (g)

Because the rise time and fall time of the gradients is dependent on the G1 value, varying G1 does not normally produce good results.

5.5 Recommendations

OIMBL recommends that D3 is varied for diffusion experiments for the following reasons:

- Provided D3 is greater that a certain minimum value (the time it takes for the gradient to reach maximum) all the fall times of the gradient pulses will be the same.
- The echo amplitude is only dependent on the effective diffusion time, not the T_1 of the sample.
- D3 has a relatively large effect on the magnitude of the effective diffusion time, allowing lower G1 values to be used and therefore smaller D2's.

Although the RINMR VB script .GETDIFF changes D3 to increment the magnitude of the Q steps, there is no reason why it cannot be easily modified to change D4 or G1 if the user prefers.

5.6 Making measurements with the GETDIFF script

A VB script called GETDIFF has been written to facilitate the acquisition of diffusion data. More information on the GETDIFF script may be found in the RINMR Pulse Sequence and Script manual. Information on how to modify the GETDIFF script to increment D4 (or G1) rather than D3 may be found in the RINMR Pulse Programming Manual.

When GETDIFF runs it prompts the user for a list (this should be an RINMR list file which contains the different values of D3 that will be used) and also a file prefix for the data files to be saved to.

While GETDIFF runs it plots the value of δ (D3) vs. the echo amplitude (note that this should not be a straight line as only the echo amplitude and not the logarithm of the echo amplitude is plotted).

Once the script has finished the data may be analysed using RI Diffusion.

5.7 Analysing diffusion data using RI diffusion

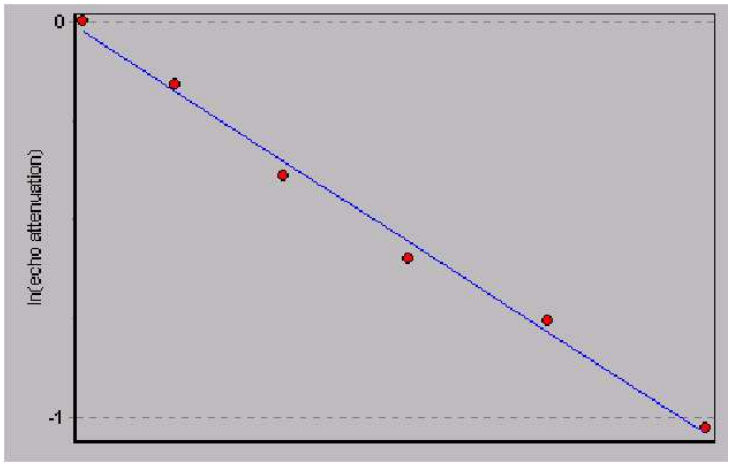
OIMBL has a separate software package for analysing diffusion data called RI Diffusion. Information of how to use RI Diffusion may be found in Chapter 16 of the RINMR user manual.

Click on the 'Load files' button and load in data acquired with the GETDIFF script. Once data has been loaded check the box next to the correct gradient (x, y or z). The program contains nominal values for the gradient strengths and amplifier currents for the x, y and z axes. These may be changed by editing the RINMR.ini file (see the Appendix in the RINMR user manual).

5.8 Hints and tips on acquiring diffusion data

Diffusion constants are highly temperature sensitive. It is essential that samples are temperature stabilised before diffusion constant measurements take place. Diffusion measurements are highly sensitive to motion. Large D4 values increase the probability that the system will be subject to vibration during the timescale of the experiment.

Calibration of the gradients should always take place before making diffusion measurements using a water sample at a known temperature. The RI Diffusion program allows the user to perform the calibration by clicking on the calibrate button and entering the water temperature. Pure water should always produce a straight line when the logarithm of the echo amplitudes is plotted against effective diffusion time, and is a good test that the instrument is working properly (see below).



The following parameters can be used as a starting set for measuring water on 10mm VT probes:

D2: 7000 us
D1: 100 us
D3: Vary between 500 and 3000 us
D4: 20000 us
GY: 32767
G1: 3000-5000

Note that these parameters may need slight modification for 18mm VT systems.

DIFFA (a more advanced program) allows the user to place a second crusher gradient pulse after the second 90º pulse. This removes any residual coherence in the transverse plane which reduces the effect of stimulated echoes. The parameter G2 controls the amplitude of the crusher pulse, which should not be greater than 500 units (when GY is set to 32767). DIFFA also allows the user to extend the data acquisition period to characterise the stimulated echoes using the parameter C1. The MARAN Ultra Pulse Programming and Pulse Sequence manual contains more information on the DIFFA pulse sequence. Note that the GETDIFFA script may be used to perform repetitive acquisitions with DIFFA.

Sample length is critical for VT experiments. If the sample is too long convection currents will be set up due to the temperature gradient across the sample. If the sample extends outside the linear region of the gradients fold back effects may occur which affect the quality of the measurements. In general it is advisable to keep the sample length as short as possible along the Y axis (the length of the tube).

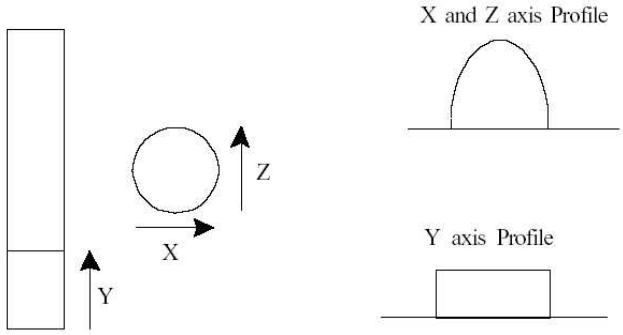
Always set DS (dummy scans) to 2 before performing diffusion experiments.

The stimulated echo pulse sequence for diffusion measurements is described in the following paper:
J. E. Tanner and E. O. Stejskal, *J. Chem. Phys.* 49, 1768 (1968).

Chapter 6 1D Profile Experiments using PROFILE

6.0 Introduction

1D profiles can be acquired using the MARAN Ultra instrument using the PROFILE pulse sequence. The profile pulse sequence will resolve the NMR signal spatially along the specified axis (usually the Y axis (or along the NMR tube) in most MARAN Ultra instruments).



The results of profiling a small rectangular cylinder of sample (for example a liquid) in the bottom of an NMR tube can be seen above. The Y axis profile is rectangular, the X and Z profiles are parabolic (note *not* semicircular). The X and Z profiles are symmetric by sample translation about the y axis.

In a homogenous magnetic field all similar nuclear spins within a sample will resonant at a similar frequency. Any NMR experiment performed in a homogeneous magnetic field will produce a bulk signal from all spins within the sample.

To impose spatial information, it is necessary to make spins in different positions in the sample resonate at different frequencies. If the frequency variation as a function of position is known, the spatial variation in spin density may be established via a process known as a Fourier transform, which tells us which frequencies are present in the data and to what amplitude.

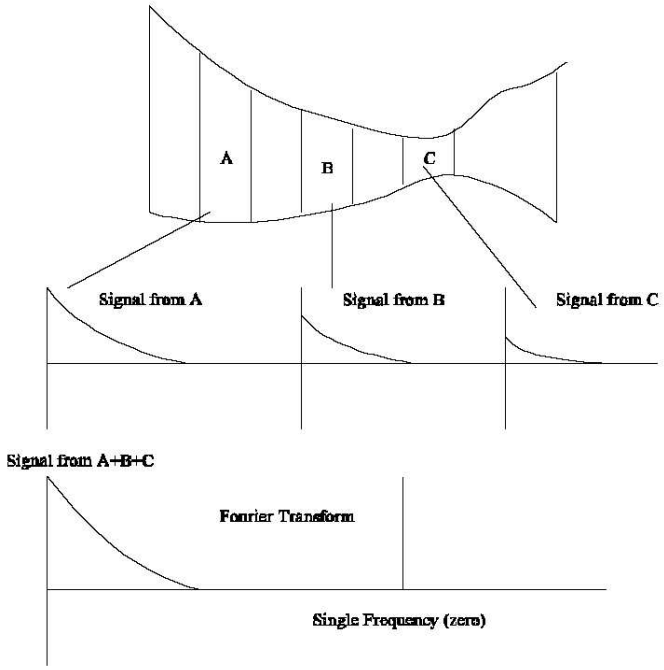


Figure 1

The principle is illustrated in Figures 1 and 2. Figure 1 shows a sample which contains NMR active nuclei in three different zones, A, B and C. The ratio of nuclei in each zone is 3:2:1. When no magnetic field gradient is applied, the situation is as shown in Figure 1. The spins from all zones resonant at similar frequencies, the total NMR signal acquired is the sum of the signals from each separate zone. When the signal is Fourier transformed, a single frequency is observed (0) as the system is on resonance. No spatial information is obtained.

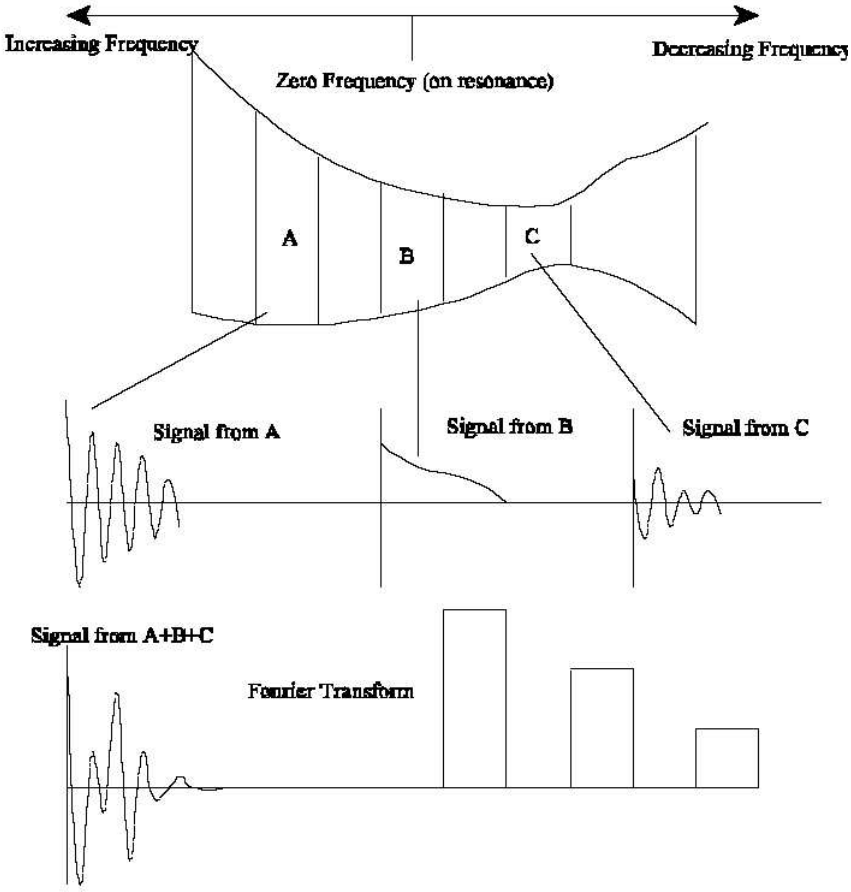


Figure 2

Figure 2 shows the result of applying a magnetic field gradient. Spins in all three regions resonate at different frequencies. The frequency at which the spins resonate is directly proportional to the zones' displacement. The total NMR signal is the sum of all three frequencies added together. The Fourier transform yields the frequencies present in the time domain data, the amplitude variation reflects the variation in the number of spins in each zone. A full mathematical description of the relationship between the 1D profile and the NMR parameters used to acquire it is beyond the scope of this document. However we can make the following statements:

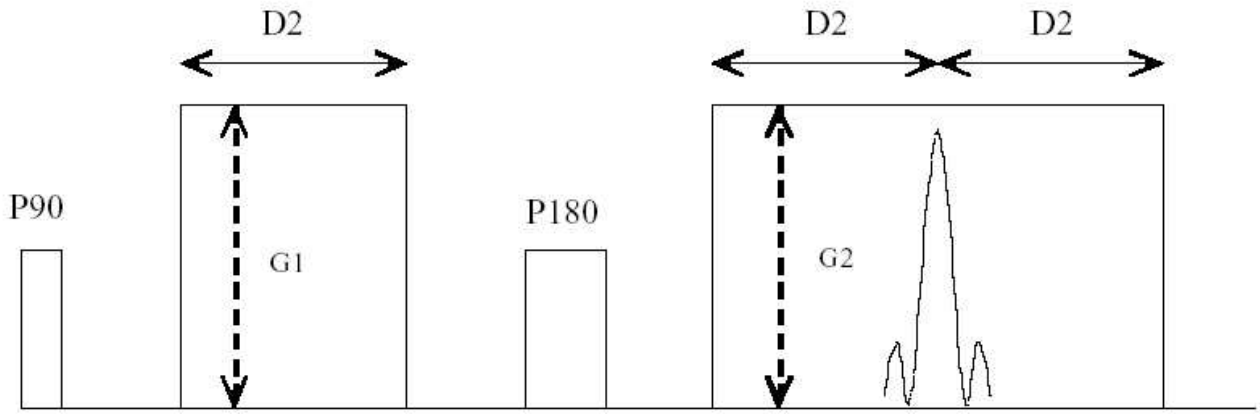
The resolution of the 1D profile is dependent both on the strength of the magnetic field gradient and the time that the spin system takes to evolve under the action of the magnetic field gradient (the time that the gradient is applied for).

The extent that the 1D profile will occupy in the Fourier domain is dependent on the total range of frequencies that are acquired within the NMR experiment, or the acquisition bandwidth, which is inversely related to the dwell time.

In practice, 1D profile experiments are not performed in constant magnetic field gradients, they are performed using pulsed magnetic field gradients. This is due to the fact that if an RF excitation pulse is applied in the presence of a magnetic field gradient the pulse becomes bandwidth limited or slice selective. This technique may be used to perform slice excitation in 3D imaging sequences.

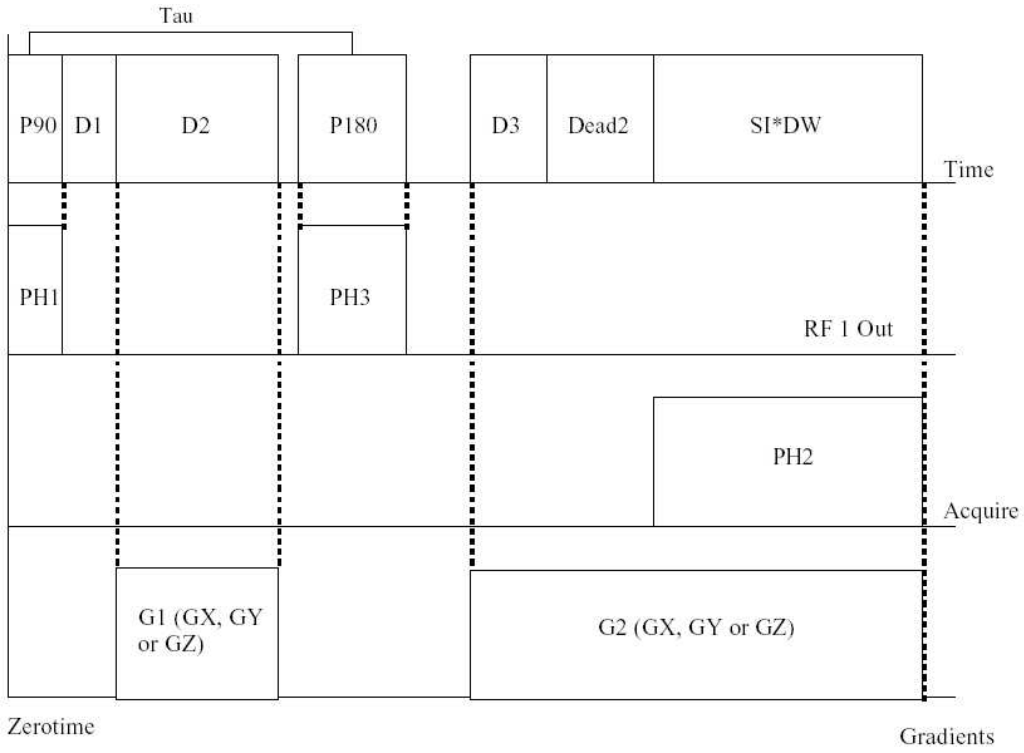
The 1D profile is rarely performed using an FID experiment in conjunction with a pulsed field gradient. This is because of artefacts that appear due to the loss of signal during the RF pulse dead time, plus other artefacts that are beyond the scope of this document.

1D profile experiments are performed using a Hahn echo experiment shown in the schematic below. Two gradient pulses occur, the echo acquired during the second gradient pulse is acquired and Fourier transformed to produce the final 1D profile.



More information on the theory regarding the acquisition of 1D profiles can be found in the books by Callaghan and Hills.

6.1 Setting up the PROFILE pulse sequence using RINMR



Note: All systems require different setup parameters to function correctly. The parameters given are guidelines for demonstration purposes please contact OIMBL for advice if necessary
To produce a 1D profile, first set the values for P90, P180, O1 and RG correctly. Information on how to set these parameters may be found in Chapter 2. Set RD to a suitable value (approximately 1S in the case of vegetable oil).

Load the PROFILE pulse sequence and use the following parameters:

D1: This is the time after the 90º pulse that the gradient is applied. Set this value to a nominal 100 microseconds.

P90: This is the length of the hard 90º pulse in microseconds. This should be set to the current 90º pulse length for this instrument.

D2: This is the length of the first (dephase) gradient pulse in microseconds. Set this to 2000 us.

D3: This is the pre-acquisition delay time which governs the time before the data are acquired. Set D3 to D2/2 (1000 us).

TAU: The distance between the 90 and 180º pulses. Set this to 4000 microseconds. Note that TAU must be greater than (D2+D3+P90/2+P180/2).

G1: The first gradient pulse amplitude. Set this to 0.

G2: The second gradient pulse amplitude. Set this to 0.

GY: The gradient global scalar. Set this to 32767.

P180: The duration of the 180º pulse in microseconds (twice the 90º pulse will suffice).

DW: The dwell time in microseconds. Set this to 15.6 us for this example, or D2/(SI).

SI: The number of points in the 1D profile, set this to 128.

FW and Dead2: Set to 1 MHz and 3 microseconds respectively for this demonstration.

Ensure PH1 is set to 02, PH2 to 02 and PH3 to 11.

Set NS to 1 and run the pulse sequence using GS. A broad echo should appear, with its maximum centred on the acquisition window. For some systems the echo may be so broad it appears as a straight line.

Increase G1 and G2 in small increments (say 25, keep the values for G1 and G2 identical). The echo should narrow.

Increase G1 and G2, making sure the centre of the echo (the highest point) stays in the centre of the acquisition window. If the echo moves off centre increase/decrease D3 to bring the echo back into the centre. Note that ideally G1 must be kept approximately equal to G2 for the echo to be centred correctly using the above parameters.

FT the echo interactively to produce a 1D profile (using the RINMR View menu). Note NS should be set to 2 or more to remove the DC spike in the centre of the profile.

To save the FFT data to disk use the FT command in process mode.

6.2 Notes on acquiring one-dimensional profiles

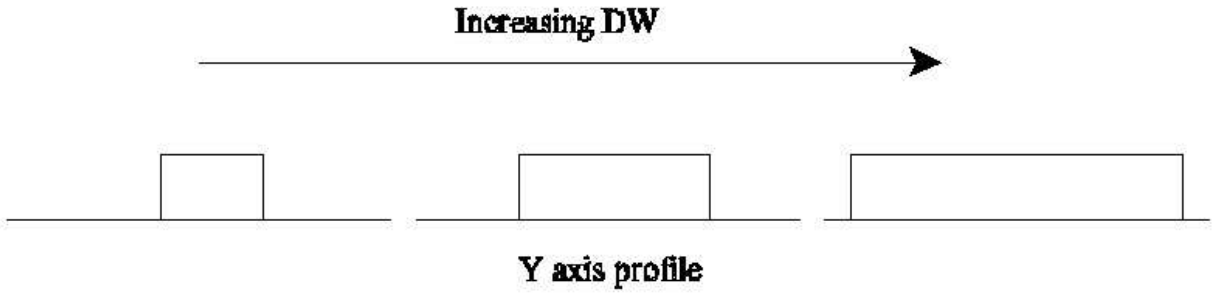
Profile experiments require much less gradient than diffusion experiments. Start off by acquiring the echo with no gradients (G1 and G2 equal to 0) and increase the gradient strength slowly until the echo narrows.

Note that if the sample has a short T_2, you must make tau shorter and in turn reduce D2, resulting in an increase in G1 and G2 necessary to maintain a similar spatial resolution.

Setting DS to 1 or more can sometimes improve the quality of the profile.

The relationship between the acquisition parameters in the time domain (G2, DW and SI) and the profile appearance in the frequency domain is complex. A qualitative discussion is given below:

The reciprocal of DW (the spacing between points in the time domain) governs the acquisition bandwidth (the range of frequencies scanned) in the frequency domain. Increasing DW decreases the acquisition bandwidth and decreases the range of frequencies scanned.



Note that the width that the profile occupies in the frequency domain (in Hz) remains constant (this is determined solely by the gradient strength). Usually the acquisition bandwidth should be altered such that the signal intensity occupies nearly all of the scanned frequencies to give the best digital resolution. The signal to noise ratio of the 1D

profile may be improved by applying a filter equal to the acquisition bandwidth (1/DW). For example when using a DW of 100 us, a 100 KHz filter will ensure that optimum signal to noise ratio is achieved. Applying a more severe filter may result in distortion of the profile.

Increasing the gradient strength (G2) increases the range of frequencies that the profile will occupy and therefore increases the image resolution at the expense of signal to noise ratio. In general using the lowest gradient strength possible and the lowest acquisition bandwidth will result in the best signal to noise ratio. However it should be noted that the gradient strength used should dominate over the magnet homogeneity or else the profile will be distorted.

6.3 Calibrating the gradient strength using a 1D profile

In order to produce quantitative diffusion measurements the strength of the gradients must be calibrated. This may be done using several methods, including using a 1D profile. This section describes how to calibrate the magnetic field gradient strength using a 1D profile.

6.3.1 Preparing and measuring the calibration sample

Firstly prepare a sample of oil of known length (l). Use a 10mm sample for a 10mm probe and 20mm sample for 18 and 26mm probes. Note that the profile will not appear rectangular but will be rounded at the top end due to the meniscus (the base of the 10mm tube is also rounded, so 1D profiles resulting from 10mm tubes have a rounded base as well). The rounding constitutes the major inaccuracy in calibrating the gradients using a 1D profile, as it makes the length of the calibration sample difficult to measure accurately.

Place the sample in the magnet and run a 1D profile experiment as described. Once you have set up the acquisition parameters and achieved a good quality profile note down the value of G2 (G2) and save the profile data to disk. Ensure the gradient scalar (GX, GY or GZ) is set to 32767.

6.3.2 Acquiring and processing the calibration data

Once the 1D profile data has been acquired Fourier transform the data using the FT command and produce the magnitude profile by typing MAG. Measure the width of the sample in Hz (following the FT the y axis of the RINMR data window is in Hz units, use the zoom function to establish the width of the profile as accurately as possible). Note down the width of the profile in Hz (f).

6.3.3 Calibrating the gradients

The profile width in Hz (f) gives the gradient strength in units of Hz/sample length/G2 gradient units.

The required value of gradient strength in G/cm can be calculated as follows:

First, convert the frequency units (Hz) to magnetic field units (gauss) using the following expression:

$$B = \frac{2\pi f}{\gamma}.10000$$

where γ is 267538030 in SI units for hydrogen atoms and the factor of 10000 *B* converts T (Tesla) to G (Gauss).

This conversion produces the gradient strength in G/sample length/G2 gradient units.

Next divide B by the sample length to produce the gradient strength in G/cm/G2 gradient units and finally divide by G2/32767 to produce the final gradient strength in G/cm. Note G/cm can be converted to T/m by dividing by 100.

The final expression is:

$$g = \frac{7.695 f}{G2 * l} \text{ G/cm.}$$

Once the gradients have been calibrated you should write down the values in the table in the appendix for future reference.

Note that the gradient calibration calculation can also be performed using the GRADCALIB script. A description of how to use the script can be found in the RINMR Pulse Programming and Pulse Sequence manual

Once the gradient calibration values are known they may be inserted in the RINMR.ini file for use in diffusion experiments. The RINMR.ini file resides in the \RINMR\bin directory and contains system specific information. The gradient information is contained under the [HARDWARE] section. The NgradStrength parameters (where N is the gradient axis, X, Y or Z) contain the gradient strengths in T/m/Amp units. The NMaxCurrent parameters contain the maximum output current of the gradient amplifier attached to the N axis in Amps. These parameters are used by RI Diffusion to calculate the self diffusion co-efficient. If these parameters are set correctly, the gradient correction factor produced by RI Diffusion will come out to approximately 1.

Chapter 7 Setting Pulse Lengths using Train90 and Train180

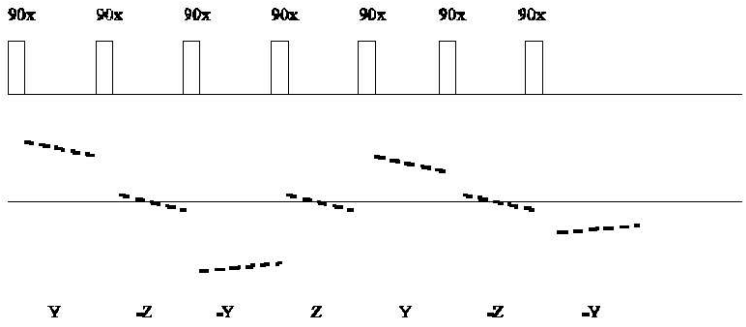
7.0 Introduction

Although the .AUTOP90 script can be used to set up the instrument P90 and P180 values, these values are approximate and under certain circumstances can lead to distortions in acquired data (for example CPMG sequences). The TRAIN90 and TRAIN180 pulse sequences enable the user to set up the 90 and 180 degree pulse lengths more accurately.

7.1 The TRAIN90 pulse sequence

The TRAIN90 pulse sequence has the following parameters:

P90	90 degree pulse length
C1	number of 90 degree pulses in the train
SI	number of points acquired after each 90 degree pulse
DW	dwell time of acquired points
RD	Repetition time
PH1	90 degree phase list
PH2	receiver phase list
Dead1	pulse dead time
Dead2	filter dead time
NS	number of scans



The TRAIN90 sequence applies C1 90º pulses to the sample. The magnetisation is rotated alternatively into the transverse plane, then along the + and - longitudinal axis. This results in the display shown above in the RINMR data window. The letters give the orientation of the magnetisation vector in the rotating reference frame. If the display is set to magnitude mode, alternate maxima and minima in the signal are observed when the 90º pulse is set correctly. The maxima and minima are modulated by an exponential envelope due to the T_1 of the sample.

As the 90º pulses are of identical phase, any error in the 90º pulse tip angle will be cumulative, leading to a trace that diverges rapidly from that indicated above.

To tune the 90º pulse length accurately first set up the P90 using the .AUTOP90 script. Next run TRAIN90 pulse sequence on a sample with C1 set to 16. Ensure that NS is set to 4 to remove quadrature alignment and gain artefacts. Vary the P90 in small increments until a suitable trace is obtained. As the sequence is very sensitive, it is often impossible to obtain perfect data. Sometimes for short values of P90 RFA0 (the pulse B1 amplitude value scalar) will also need to be altered to provide additional resolution.

7.2 The TRAIN180 pulse sequence

The TRAIN180 pulse sequence is operated in a similar manner to the TRAIN90 pulse sequence. The pulse train consists of C1 180º pulses. Using this sequence when the P180 value is set correctly the magnetisation is rotated

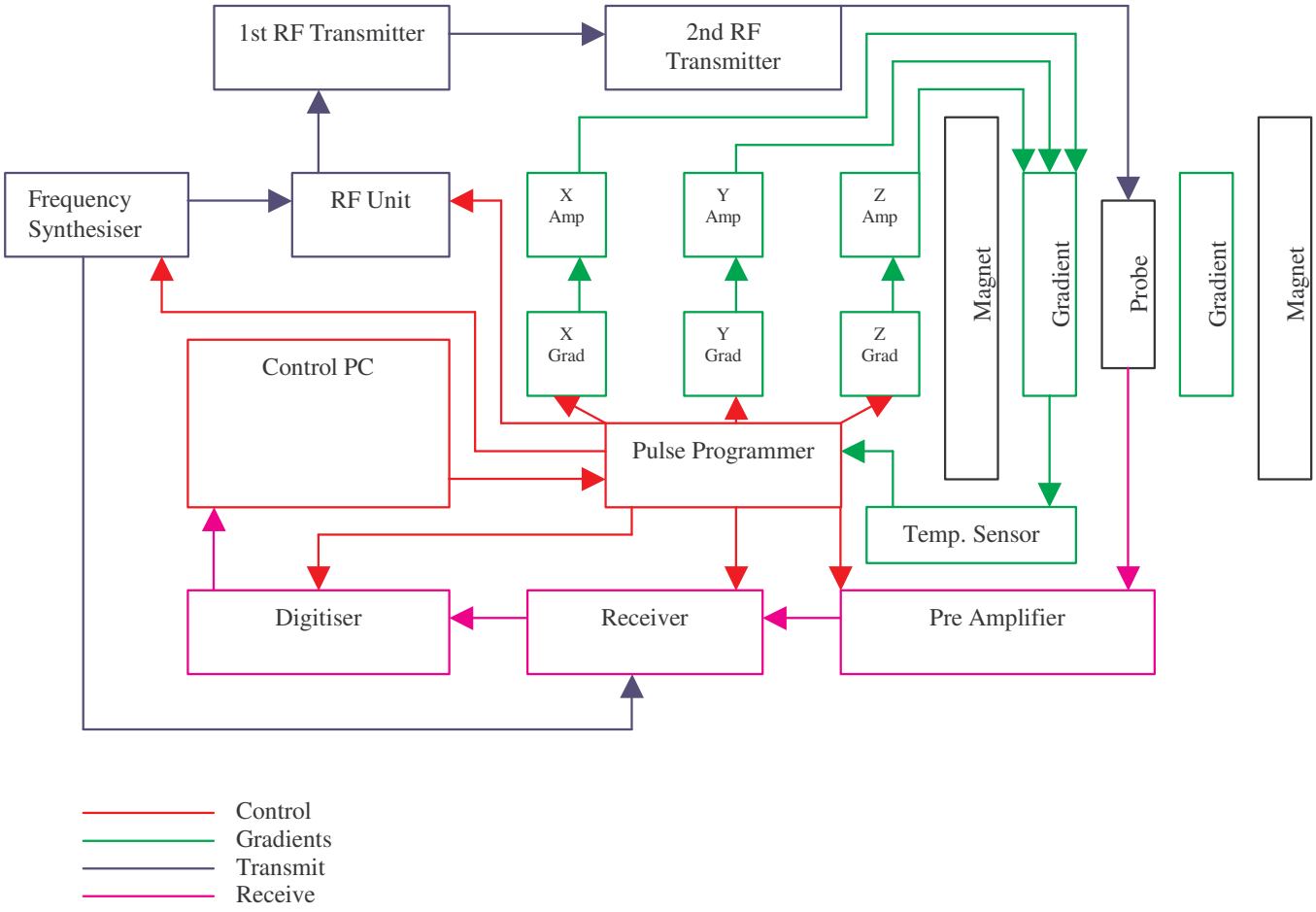
alternatively along the positive and negative longitudinal axes and does not appear in the transverse plane, so no signal is observed.

Chapter 8 Introduction to NMR Hardware

8.0 Introduction to NMR hardware

The purpose of this chapter is to describe the main components of an NMR spectrometer and how they interact. It is designed for the non expert user/electronic engineer, although the complexity of the hardware means that users require some familiarity with basic electronic engineering concepts in order to fully understand some aspects of the description. The information is not intended for expert users wishing to understand in depth the workings of the MARAN Ultra spectrometer or users wishing to interface their own hardware to the MARAN Ultra system. Users requiring further technical information should contact OIMBL for advice.

8.0.1 Schematic of NMR spectrometer component layout



8.1 System component description

8.1.0 Control PC

The control PC is used as an interface to the pulse programmer and as a data display device. MARAN Ultra spectrometers communicate with the PC via an Ethernet link, which serves both as a link to download instructions to the pulse programmer and an uplink to read back the acquired data from the ADC's.

8.1.1 Pulse programmer

The pulse programmer controls the sequence of events (RF, gradient and acquisition) which will occur during the NMR experiment. MARAN Ultra pulse programmers have over 500,000 possible events with a 50 nanosecond timing resolution per event. The pulse programmer performs its tasks via a series of control lines. For example, 1 line is linked to the RF transmitter enable and this line is toggled to enable the transmitter. Other lines are linked to the frequency synthesizer, the ADC's, the RF Unit and the digitiser.

The MARAN Ultra pulse programmer can control over 256 data lines, of which y are routinely used by the NMR hardware. Control of the gradient boards is performed via a sequential bus.

8.1.2 Frequency synthesiser

The frequency synthesizer generates the sinusoidal waveform at the NMR frequency (SF+O1) which is subsequently amplified by the RF transmitters. MARAN Ultra synthesisers produce the NMR frequency (usually in the range 1-30 MHz) by direct digital synthesis (DDS). Two waveforms, a sinusoid and a cosinusoid are synthesised. Mixing of the two waveforms can produce an output waveform of any required phase. As well as the transmit signal, the frequency synthesizer also produces a reference signal for the receiver which is used to demodulate the NMR signal prior to acquisition.

8.1.3 RF unit

The RF unit produces the RF pulses which may be amplitude and phase modulated for selective excitation experiments. The RF unit produces an industry standard 0dbm output signal, which may be interfaced to most common RF amplifiers.

8.1.4 The RF transmitters

The RF transmitters have two input signals, a blanking control line from the pulse programmer which ensures that the output from the RF transmitter is zero when no output pulses are required, and the signal input from the RF unit. MARAN Ultra RF transmitters must be enabled 20 microseconds before an RF pulse is required. Several amplifier combinations are possible, a 25W amplifier is used as the first stage, usually driving a 300W or 600W amplifier as the second stage. The RF transmitter power affects the 90º pulse length, which is inversely proportional to the square of the transmitter power.

8.1.5 X, Y and Z gradient boards

The gradient boards contain DAC's which produce the industry standard 5V input signals to the gradient amplifiers. These may be simple square pulses or more exotic gradient shapes such as sinusoids. The gradient boards are connected to the pulse programmer via the sequential bus, which is used to download gradient shapes to the gradient boards.

8.1.6 X, Y and Z gradient amplifiers

These amplifiers produce the output current for the magnetic field gradients from the 5V input signal produced by the gradient boards. The gradient amplifier performance is dependent on the maximum output current of the amplifier (maximum gradient strength available), the maximum voltage output of the gradient amplifier (governs the rise time of the gradient pulse) and the gradient amplifier slew rate or bandwidth (governs the rise time of the gradient pulse).

8.1.7 Magnet

The magnet generates the Bz field necessary for generating the population inversion that allows NMR experiments to be conducted. The ideal NMR magnet has the following properties:

1. As homogeneous as possible over the defined sample volume. The MARAN Ultra magnet homogeneity varies depending on magnet frequency and type.
2. As strong as possible (gives the best signal to noise ratio, although in certain special situations this is not necessarily the case).

3. Have a high magnetic field stability as a function of time. Permanent magnets have a high coefficient of variation of magnetic field strength as a function of temperature, so it important to take as many steps as possible to stabilise the magnetic field.
4. Have a low stray field.

8.1.8 Gradients

The gradients are used to generate linear variations in Bz as a function of X, Y and Z ordinate across the sample in order to produce spatial or translational information.

An ideal gradient should have the following characteristics:

1. Be as strong as possible - the stronger the gradient, the less current required for a given gradient strength.
2. Have the shortest rise time possible - if the rise time is long the gradients cannot be switched rapidly and the gradient pulses will not be square.

MARAN Ultra systems are available with actively shielded and unshielded gradients. The addition of the active shielding decreases the gradient rise time at the expense of gradient strength. Many of the MARAN Ultra 2 MHz systems also allow the user to short out the active shield, which increases the gradient strength at the expense of rise time.

Due to the finite resistance of the gradients and the large currents produced by the gradient amplifiers, considerable heating may occur in the gradients. Water cooling is used to reduce the operating temperature of the gradients, and the temperature of the gradient is continuously monitored by sensors embedded in the gradient coils (see section 8.1.10).

8.1.9 RF Probe

The RF probe transmits the RF pulse to the sample and receives the NMR signal. Section 1.4 contains more information on RF probes.

8.1.10 Gradient temperature sensor

The temperature sensor monitors the temperature of the magnetic field gradients during gradient experiments. If the temperature exceeds a certain level, the experiment will be stopped.

8.1.11 Pre amplifier

The pre-amplifier amplifies the NMR signal from the microvolt to the volt level. Pre amplifiers should have as high gain and as low noise figure as possible. Pre amplifiers must also be able to withstand the high voltages produced by the RF transmitter, although steps are taken to protect them from this.

MARAN Ultra pre amplifiers have a three stage amplification and their dead times are less than 5 microseconds. The amplifiers are broadband, 1-500 MHz.

8.1.12 Receiver and quadrature detection

After the signal has been amplifier it is demodulated via the reference signal sent from the frequency synthesizer and detected in quadrature and filtered by the receiver prior to digitisation.

MARAN Ultra systems are fitted with two filters as standard, 1 MHz and 100 KHz. Systems with high performance filters fitted can use continuously variable filters from 1 KHz to 1 MHz in 100 Hz steps. Care must be taken to ensure filters are set correctly - too severe a filter leads to modulation of the NMR signal by the filter - to wide a filter may lead to sub optimal signal to noise ratios.

The use of quadrature detection allows the detection of the complete NMR signal while preserving phase information (necessary for inversion recovery T_1 experiments).

Quadrature detectors are subject to a number of artefacts which may be removed via the use of a data acquisition scheme known as phase cycling. Artefacts that are present are as follows:

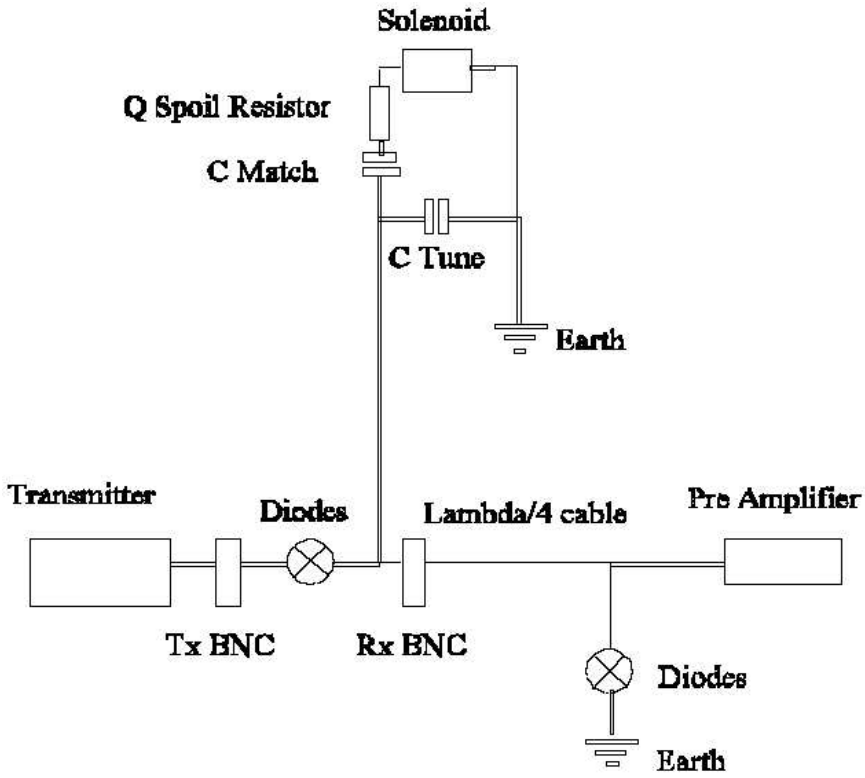
- DC levels may be present on both quadrature channels, resulting in NMR signals that do not decay to zero.
- The gain of one quadrature channel may be greater than the other.
- The quadrature channels may not be orthogonal.

The artefacts created by the above may be removed by ensuring that the NMR detection and excitation is performed along each of the axes of the rotating frame in turn. The cancellation of the above quadrature artefacts can be verified using simple vector addition.

8.1.13 Digitiser

The digitiser records the data produced each of the quadrature channels. The shortest digitisation time for the MARAN Ultra instrument is 0.1 microseconds (10 MHz). The ADC's within the digitiser have 12 bit resolution and average to a 32-bit store.

8.2 The transmit/receive circuit



An illustration of a typical transmit/receive circuit is shown above.

The BNC connections on the MARAN RF probe are shown as reference points.

Signal from the transmitter passes through crossed diodes before entering the probe circuit. This prevents noise from the transmitter circuit propagating to the pre-amplifier while the transmitter is switched off.

The transmitter drives the probe circuit, which is tuned to the resonant frequency of the magnet (via C tune) and matched to the output impedance of the transmitter (50 ohms) (via C match) to ensure all the RF power is

transmitted to the probe. The Q spoil resistor lowers the Q of the probe circuit (see section 8.3.1) and absorbs much of the RF power.

The receive signal passes out of the probe via the Rx BNC and is linked to earth via a pair of crossed diodes to protect the pre amplifier while the RF pulse is being applied. The lambda/4 line between the pre-amplifier protects the pre-amplifier from reflected power caused by mismatch between the output impedance of the transmitter and the probe circuit. Due to the extreme lengths of lambda/4 cables at low frequencies (at 2 MHz the lambda/4 cable is approximately 20 metres long) this element is often replaced by a lumped array.

8.3 RF probes

8.3.0 Design considerations for RF probes

RF probes are a key component in any NMR spectrometer. Careful optimisation of RF probe design can lead to significant gains in spectrometer performance. However, it should be noted that RF probe performance is very much a compromise between several factors. The ideal RF probe has:

- A short 90º pulse length (P90).
- As short as possible a dead time (Dead1).
- As high as possible sensitivity.
- A high level of robustness.

Many of these requirements are orthogonal, for example it is impossible to decrease the RF probe dead time while maintaining the same pulse length.

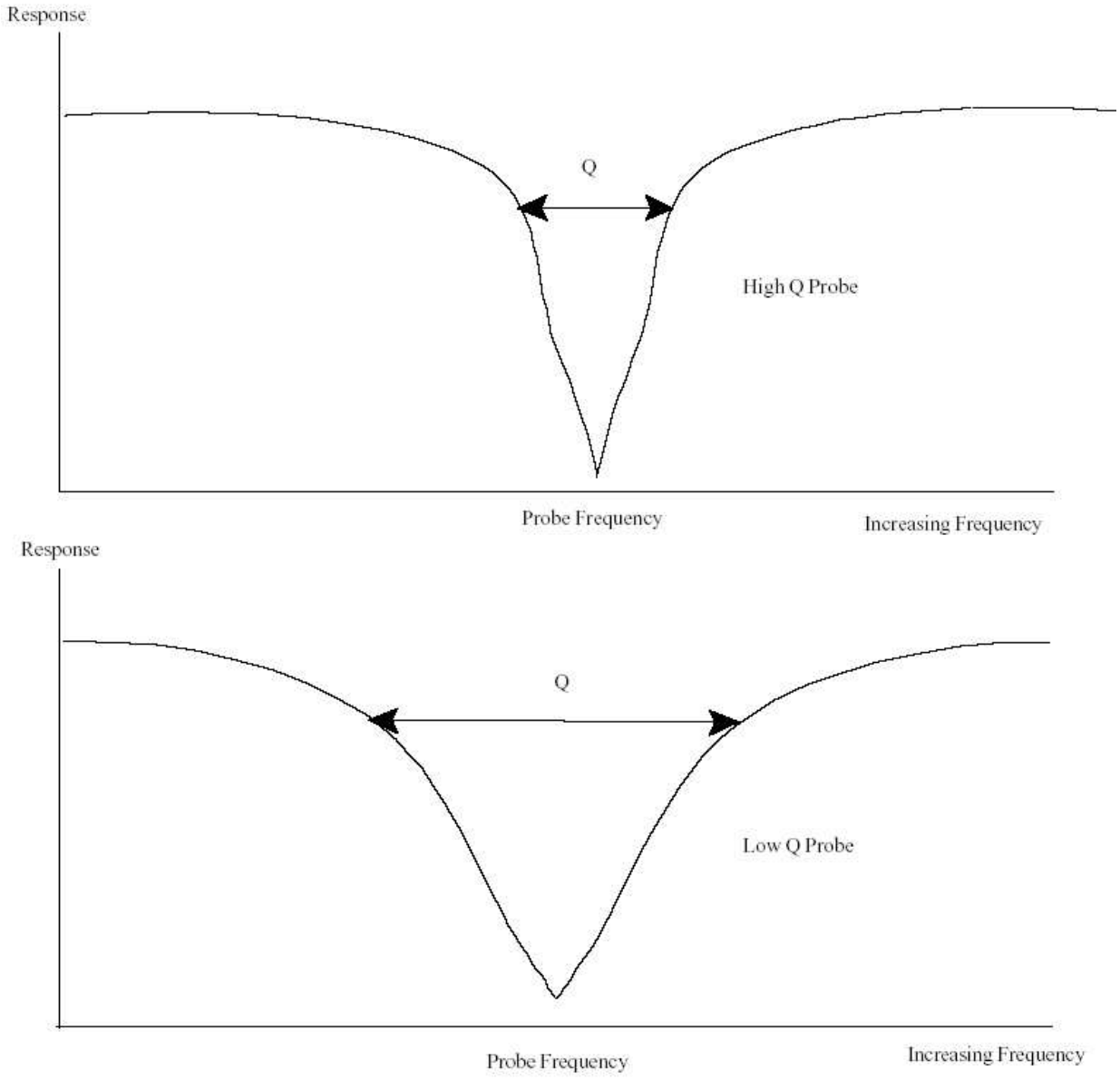
In addition, RF probes must have the following physical characteristics:

- The probe geometry must generate a B1 field orthogonal to the Bz field produced by the permanent magnet, while allowing easy sample access. The B1 field generated should be as homogeneous as possible over the sample volume.
- The probe must be mechanically robust as a lack of secure mounting may lead to an increase in the dead time caused by mechanical vibration.
- The probe must be constructed so no background signal is observed from the probe itself (for example the probe former on which the solenoid is wound cannot contain any 1H atoms).

8.3.1 Characterising RF probe performance

RF probe performance is usually characterised by a single parameter know as the RF probe Q. The RF probe Q (or quality factor) can be thought of as a measure of the range of frequencies (or bandwidth) that a given RF probe will respond to.

The diagram below shows a low Q and a high Q RF probe and their response as a function of frequency. High Q probes have a relatively narrow response bandwidth, their 90º pulse lengths are relatively short and their signal-to-noise ratios are relatively high. Dead times of high Q probes are relatively long. High Q probes are relatively susceptible to loading by the sample (placing a sample in the probe detunes the coil easily) and due to the extremely high voltages across the tuning and matching capacitors tend to be less robust than low Q probes.



In contrast low Q probes have a relatively wide frequency response bandwidth, have relatively long 90º pulse lengths, relatively low signal to noise ratios and relatively short dead times. They are relatively insensitive to detuning by the sample and due to lower voltages across the tuning and matching capacitors are more robust than high Q probes.

8.3.2 Specifying probe Q for benchtop instruments

The best configuration for RF probe Q depends on the application. Liquids, which have slowly decaying NMR signals can be analysed without the requirement for short dead times. Therefore many liquid state high field spectrometers have high Q probes to achieve the best possible signal to noise ratios. Benchtop NMR instruments are normally equipped with relatively low Q probes for the following reasons:

Bench top instruments are often used to measure solid signals so a short dead time is important. The increase in pulse length caused by moving to a lower Q can be compensated for by increasing the RF transmitter output power. Bench top instruments are designed to measure large numbers of samples continuously and for long periods in quality control type applications and as a consequence require a high level of robustness.
Because NMR signals from solids decay rapidly they have a high bandwidth. The RF probe must have a correspondingly high bandwidth to both excite and detect all frequencies present within the NMR signal.

Most bench top NMR systems (apart from rock core analysers) are not equipped with RF probe tuning apparatus (wobble box). Thus the probe must accept a wide range of different sample types without being significantly detuned.

In practice constructing RF probes with low enough values of Q using the RF probe geometry alone is not possible. To lower the RF probe Q, a resistor (know as a Q spoil resistor) is connected in series with the RF probe coil. The Q spoil resistor has a high power rating, as often a significant amount of the RF power generated by the transmitter is dissipated by it.

8.3.3 Tuning probes using the wobble box

Some bench top spectrometers (particularly rock core analysers) have a tuning facility known as a wobble box to allow the user to tune the RF probe.

The wobble box sweeps through frequencies about a specified value and records the response of the RF probe. The difference between the resonance frequency of the magnet and the best match of the probe to 50 ohms can be observed. The probe tuning capacitor C tune can be varied until the resonant frequency of the probe matches the resonant frequency of the magnet.

8.4 Pulse sequence execution

The execution of a pulse sequence can be thought of as the following steps:

1. Download Pulse sequence from control PC to pulse programmer.
2. Pulse programmer sets synthesizer frequency (SF+O1), receiver gain (RG) and filter width (FW).
3. RF Transmitter amplifier enabled.
4. RF pulse output (P90).
5. Pause for dead time of probe/pre-amp (Dead1).
6. Pause for dead time of filters; enable the receiver (Dead2).
7. Enable the receiver and activate the digitisers.
8. Acquire the data (SI *DW).
9. Download data from digitisers to the PC.
10. Display data.

The MPL (MARAN Programming Language) for the FID pulse sequence described above is as follows:

```
PROCEDURE Sequence;

BEGIN
Duration(20*us,TXEnable1);
ZeroTime;
Duration(P90,RF(PH1)+TXEnable1);
Duration(Dead1,0);
Duration(Dead2,REC);
FOR n:=1 TO SI DO
Duration(DW,ADC(PH2)+REC);
Next(PH1);
Duration(RD,0);
```

```
END;
```

Chapter 9 Tuning Probes using Wobble

9.0 Introduction

Some samples, especially those containing paramagnetic/ferromagnetic materials or having a high saline (ionic) content interfere with the tuning of the RF probe. These samples shift the best response of the probe away from the resonant frequency and cause both an increase in the 90º pulse length and a reduction in the signal to noise ratio of the probe. The effect is especially marked on probes that have high Q.

9.1 The wobble facility

Systems which are used to measure samples which are known to detune probes significantly (for example rock core analysers) are often equipped with a tuning facility to allow the user to tune the RF probe for optimum performance on each sample. This facility is known as wobble. The wobble technique excites all specified frequencies simultaneously and produces an output of the probe response.

In RINMR, the wobble process is performed using the pulse sequence WOBBLE. To load the wobble pulse sequence type:

LOAD WOBBLE

in the RINMR command box in acquisition mode.

Next the WOBBLE pulse sequence parameters should be specified. These are SF (the value of the centre line in MHz in the RINMR data window) and WW, the width of frequencies to be scanned.

For example specifying a WW of 0.5 MHz and an SF of 2 MHz will display the probe response from 1.5 MHz to 2.5 MHz.

Note: The centre frequency displayed by the wobble pulse sequence is dictated entirely by sf, not sf+o1.

To run wobble type GS1 in the command box and set the display type to magnitude using the view menu. The data window should show a V shaped trace. By changing the position of the probe tuning capacitor the optimum response of the probe (the bottom of the V shape) can be made to coincide with the centre frequency displayed by the data window (SF).

Appendix

Parameter	Factory Setting
SF	
P90	
P180	
O1	
Dead1	

Gradient Orientation	Strength T/m	Strength G/cm
X (1D profile)		
Y (1D profile)		
Z (1D rpofile)		
X (Diffusion)		
Y (Diffusion)		
Z (Diffusion)		