EFT
Quick Start Manual

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About this Manual

This manual provides a set of procedures and software commands to perform a simple, but complete, NMR experiment with the EFT spectrometer.

How this Manual is Organized

The EFT Quick Start Manual includes one chapter and one appendix.

Operation of the EFT Spectrometer

This material explains all the necessary operations to perform the first FT experiment. It describes the WinPNMR Software commands for setting experimental parameters as well as for acquiring data. It also describes basic commands for processing, and printing experimental data using the Nuts NMR data processing software.

Appendix A PNMR Help

This appendix provides a complete listing of the PNMR commands and their functions.

Conventions Used in this Manual

Fonts

The following table explains how different fonts are used in this manual.

If you see . . .	It means . . .
`Courier New Bold`	This text is displayed on the computer screen. It is also used to designate file and directory names.
`Courier New`	Text the user types that appears on the screen.
`Courier New Italic`	Variable for which the user types the value, either numeric or text. For example, if the manual reads: **`Enter filename:`** *`filename`* ... the user might type ... **Enter filename**: `ethylacetate_dept`
Square brackets [ ] Bold	The text between the brackets is an item or text in the Windows environment to be selected with the mouse.
`Angle brackets` < >	A key or combination of keys on the keyboard, such as `<Enter>` or `<ESC>`, which do not appear on the screen. When two keys must be depressed simultaneoulsy, action is indicated as `<Key+Key>`, for example: `<Alt+Tab>`.

Icons

This icon . . .	Indicates . . .
NB	Notes are information, tips or suggestions
	Important indicates an item to check carefully
	Cautions indicate hazardous situations which, if not avoided, may result in property damage or minor injury.

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OPERATION OF THE EFT SPECTROMETER

The First Proton Spectrum

The following instructions guide the first time EFT spectrometer user in obtaining a routine proton NMR spectrum of a typical organic compound. The step-by-step instructions describe acquiring a time domain proton spectrum (FID), transforming the FID spectrum to the frequency domain, correcting the phase, integrating the spectrum, labeling the spectrum and plotting the data.

1. Prepare a sample for EFT spectrometer.

Samples are prepared in 5 mm NMR tubes. A suitable concentration is obtained by dissolving five to ten milligrams of sample in 0.5 ml of a solvent. The usual solvents are carbon tetrachloride or deuterated chloroform. A small amount of tetramethylsilane (TMS) is already dissolved in most solvents used to prepare NMR samples. TMS has one sharp signal that may be defined as the zero point to which all other signals are referenced.

2. Position the spin collar on the sample tube.

NMR tubes are made of thin glass and, thus, are both very fragile and potentially dangerous. Use care when inserting NMR tubes into, or removing them from, both the depth gauge and the probe.

Proper positioning of the NMR tube in the magnet is crucial for obtaining signals from the spectrometer. The NMR tube is fitted with a collar that makes the sample tube spin and determines its position in the probe. The spin collar position is adjusted using a depth gauge located either at the front of the magnet console or provided separately. Carefully insert the bottom of the NMR tube into the spin collar and then insert the end of the NMR tube into the depth gauge. Gently push down on the NMR tube. As the tube slips into the depth gauge, the position of the collar on the NMR tube moves up. When the NMR tube touches the bottom of the depth gauge, the collar is correctly positioned on the NMR tube.

3. Insert the sample into the EFT spectrometer.

Wipe the outside of the NMR tube and the spin collar with a tissue to remove adhering particles or fingerprints. Carefully insert the NMR tube, with the properly positioned spin collar, into the probe. To insert the NMR tube, position the end of the NMR tube in the top of the probe located below the plastic door on the magnet housing. Be certain the sample eject air is flowing. Release the NMR tube, switch off the sample eject air, and allow the tube to fall into the probe. The sample tube comes to rest on a cushion of air and should spin smoothly.

Verify that the NMR tube is spinning properly. The sharpest signals are observed when the sample tube is spinning smoothly (consistent rate and without wobble) at 35-45Hz. The spinning rate can be determined by measuring the distance (in Hz) of spinning sidebands from the TMS signal. If the NMR tube does not spin properly, remove the NMR tube from the probe. Use a tissue wetted with few drops of alcohol to clean both the NMR tube and the spin collar.

4. Set the EFT operating parameters.

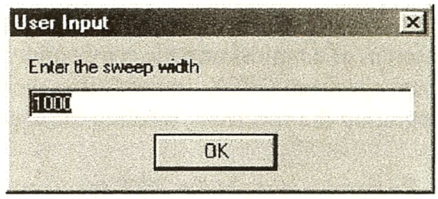
The spectrometer acquisition parameters, or settings, should be checked before collecting data. The acquisition parameters are displayed on the PNMR screen. If necessary, use the key combination <Alt+Tab> or click on the PNMR icon on the task bar to show the PNMR screen.

To change an acquisition parameter, type the appropriate two letter command. For example, to change the sweep width type

```
H1>ns<Enter>
```

NB All of the two letter PNMR commands for controlling the spectrometer must be followed by <Enter>.

In this case, the computer responds with the following dialog box



To change the sweep width to 1000, type

```
1000<Enter>  or  [OK]
```

For the purposes of this exercise, verify that the acquisition parameters are set to the values listed in Table 1. Leave all other parameters at their default settings.

Acquisition Parameter	Command	Value
size of data array	si	16384
number of scans	ns	1
receiver gain	rg	20
pulse width (90°)	pw	12

Table 1 Initial Data Acquisition Parameters

5. Adjust the receiver gain.

The receiver gain should always be checked for proper setting. The optimum value is primarily dependent upon the sample concentration. For a sample of 100% water (with a proton concentration of about 110 molar), the receiver gain should be set to a value of about 5. For a sample of 4-10 mg of a typical organic molecule in 0.5 ml of solvent, the receiver gain should initially be set to a value of 60. The best value is one that provides the maximum gain without "clipping" the signal.

To determine the proper receiver gain, observe the FID signal by typing

```
H1>gs<Enter>
```

The gs (go setup) command causes the spectrometer to pulse the sample at intervals of a few seconds and to display the FID signal. Horizontal red lines on the display indicate the maximum signal that can be digitized by the spectrometer. The FID signal appear as a damped sine wave. If the receiver gain is set too high (Fig. 1), the signal is said to be "clipped" and is displayed in red.

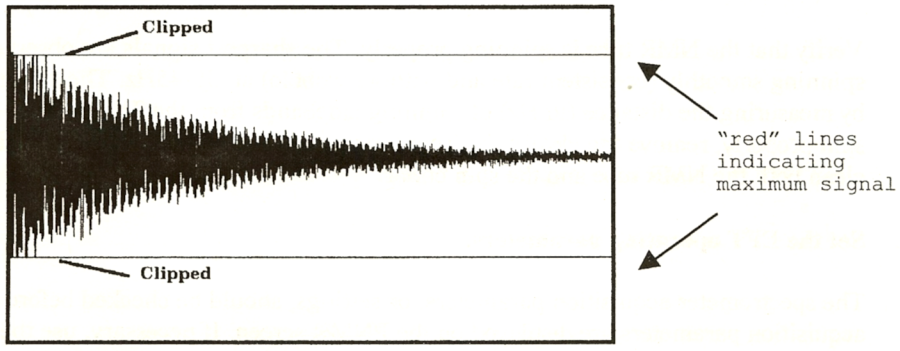
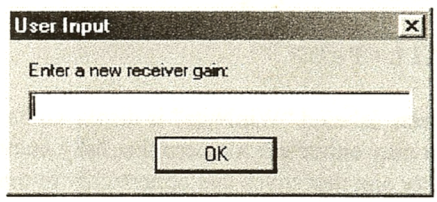


Figure 1 Signal with the receiver gain set too high.

The receiver gain can be changed, while observing the FID in the GS MODE, by pressing the key combination:

<Ctrl+G>



In the dialog box enter:

value<Enter> or **[OK]**

Change the receiver gain while observing the FID until the signal is no longer clipped (Figure 2).

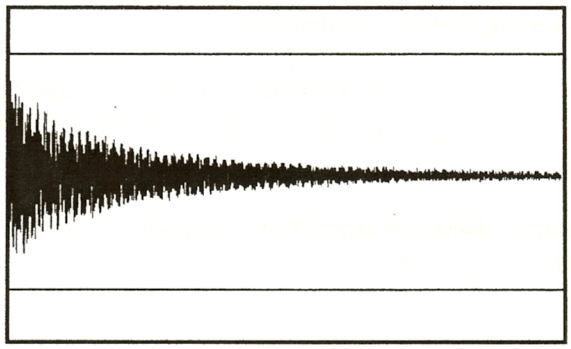


Figure 2 Signal with correct receiver gain.

Exit the GS MODE with key combination

<Ctrl+Q>.

6. Acquire data with the EFT Spectrometer.

To acquire the NMR data, type

H1>zg<Enter>

The zg (zero and go) command sets the data storage memory to zero, initiates the data acquisition software routine for the set number of scans, and automatically saves the acquired data.

NB One scan is usually sufficient to generate high quality spectra. Increasing the number of scans four-fold improves the S/N value (signal-to-noise ratio) two-fold. Thus, acquisition for 4 scans generates a spectrum with a two-fold improved S/N.

The acquired data from the most recent scan is automatically saved in the EFT folder as the file named PNMR.FID for immediate processing. PNMR.FID is temporary and is over-written by the next acquisition.

7. Process data using the NUTS NMR software.

Switch to the NUTS program with the key combination

<Alt+Tab>

The screen labeled "WinNuts" is displayed on the monitor. To process the FID data in the PNMR.FID file, you may either use a macro that fully automates the task, including printing the spectrum, or preferably one that gives you opportunity to make changes before printing.

Automatic Data Processing

Employ full, automatic processing with key combination

<Ctrl+F1>

NB Commands in NUTS should **NOT** be followed with <Enter>. Note as well that commands are not case sensitive.

The Data Acquisition Parmeters dialog box appears providing the option to enter comments, date, etc. To print the spectrum, type

<Enter> or **[OK]**

Manual Data Processing

The recommended alternative is to process the data with key combination

<Ctrl+F2>

The Data Acquisition Parmeters dialog box appears providing the option to enter comments, date, etc. To continue, press

<Enter> or **[OK]**

a. Phase correct data.

To adjust the phase correction, first enter the "Zoom" routine by typing

>zo "Zoom" may also be accessed by double clicking the left mouse button.

Use the left mouse button to select a peak on the left side of the spectrum. Place the cursor to the left of the peak, click and hold the left button, drag right until the peak is highlighted, release the button and press

<1> :identifies first phase pivot

Repeat the process for a peak on the right of the spectrum. When this peak is highlighted press

<2>

<Enter> :return to base level prompt

Enter the phasing expanded subroutine by typing

```
>pe
```

Press and hold the left mouse button while moving the mouse left (or right) until the selected peak phase is properly adjusted. Release the mouse button and repeat the process on peak 2 by using the right mouse button. Switch between the two peaks simply by pressing the appropriate mouse button. When the phase is correct

```
<Enter>      :return to base level prompt
```

b. Integrate the data.

Type the following commands

```
>mf          :sets the tallest peak in the spectrum to full scale
>fb          :for flatten baseline
>l           :initiates the baseline flattening algorithm (least squares polynomial fit
><Enter>     :exit the fit baseline routine
>id          :enter integral display
>c           :display continuous integral and thereby remove previous integral sections
```

Use the slider at the left of the screen to adjust the amplitude of the integral display. For each broken integral, click left mouse button twice on the left side of selected peaks(s) and once on the right side.

To assign a relative value for an integral, place the cursor near the displayed value, press and hold the left mouse button, simultaneously press

```
<v>
```

Enter a number in the dialog box (Fig 3).

```
<Enter>      :return to base level prompt
```

The key combination <Ctrl+I> toggles the integral display on or off, either at the base prompt or while within any other routine.

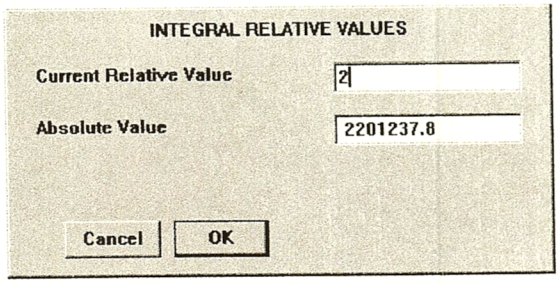


Figure 3 Integral relative value dialog box.

c. Select data peaks.

For automatic peak picking use:

```
>pp    :picks peaks with amplitudes > the MH parameter (default 10% of max.)
```

For manual peak picking use the "Define peaks" routine:

```
>dp    : cursor becomes a crosshair with a dp label.
<a>    :automatically picks peak;
<c>    :clears the peak pick;
<k>    :removes a single peak pick nearest the cursor.
```

(Add peak by clicking the left mouse button near peak.)

<t> :update peak pick table;
<Ctrl+B> :toggles peak pick table on/off;
<Ctrl+P> :toggles the peak labels on/off.
<Alt+E+I> :toggles on or off the peak information label, which corresponds
:to the first column in the peak pick table.
<Enter> :return to base level prompt

d. Print data.

Type:

>pl or **[File][Print]**

The result of the exercise is a printed copy of the FTNMR spectrum for 5% ethylbenzene with 1% TMS as reference is presented Fig. 4.

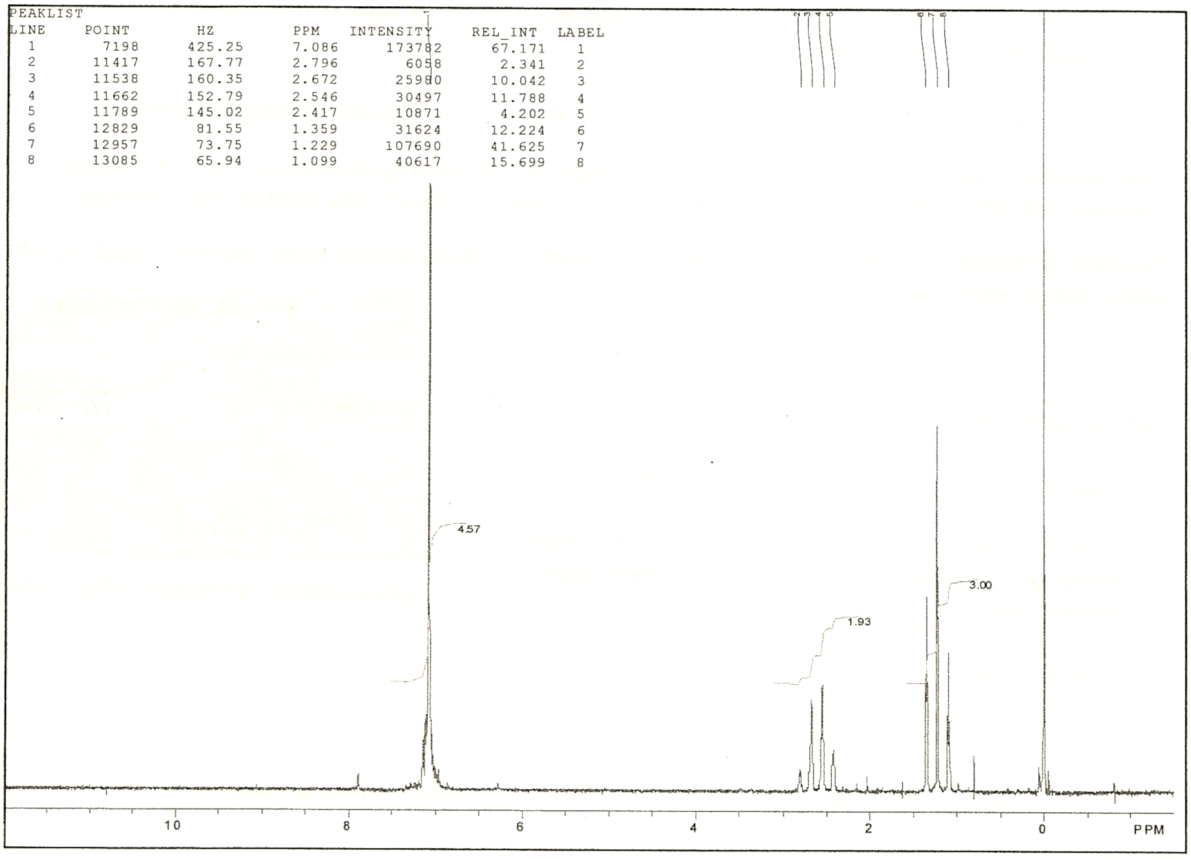


Figure 4 Proton spectrum of 5% ethylbenzene with 1% TMS.

8. Remove the sample from the probe.

Remove the sample tube from the probe. Remove the spin collar from the sample tube and place it where the next operator can easily find it.

APPENDIX A PNMR HELP

The PNMR Help file is available on-line from the Windows Desktop with the commands:

[Start][PNMR Help]

```
PNMR_HELP  2001-11-23
*************************************************************************

  GENERAL
        All commands except the <Ctrl+ > subcommands are executed
        only when followed by an <Enter>.

        To enter data type the parameter followed by a space then the
        number and Enter. For example, H1>w1 2000<Enter> will set the
        spectrum width to 2000Hz. Alternatively type the parameter
        followed by <Enter> and put the value in the dialog box.

        <Ctrl+k> halts GS, ZG or any Pulse Program immediately.
        <Ctrl+q> halts GS, ZG or any Pulse Program after the next scan.

  Arrow Keys:  Up and Down arrow keys change vertical scale.
        Right and Left arrow keys increase and decrease respectively the
        O1 in increments of 1 sweep width (w1).  Use this feature only in
        GS mode for finding strong signals.

  GS MODE (Go Setup)
        <Ctrl+g> set receiver gain (RG)
        <Ctrl+p> set observe pulse width (PW)
        <Ctrl+r> set relaxation delay (RD)
        <Ctrl+d> toggles dual FID display on/off
        <Ctrl+s> toggles between FID and spectrum

  IMPORTANT SYSTEM COMMANDS
        EX    EXIT the PNMR program.
        FO    FIELD OFFSET for changing the field in order to set a
              reference peak to particular offset, 0.0ppm in the case
              of TMS.
        RE    READ in an initialization, shim, or data file. Format;
              C13>re c13.ini<Enter>, H1>re best.shm<Enter> or
              C13>re c:\eft\data\my_fid.1<Enter>
        WR    WRITE the FID to a data file, write the current
              parameters to an initialization (.ini) file, or write
              the current shim settings to a shim (.shm) file. Without
              path, writes to the current folder or directory.

  NMR SETUP COMMANDS
        NU       Change NUCLEUS. The prompt will change to indicate
                 the nucleus selected or ask for a new nucleus. For a
                 new nucleus one must make the appropriate changes in
                 the parameters and write the initialization file for
                 that nucleus. The PNMR prompt indicates which nucleus
                 is currently being observed, for example C13>.
        PREP     A combined program to synchronize the shims, shim the
```

radial gradients spinning, then the first and 2nd order axial gradients, set the spin rate and finally roughly set the field to the right value. It is a good idea to start each morning with the PREP program. H1 only.

SHIM SHIM Y and 2nd order axial gradient. H1, F19, C13, MM and X nuclei.

DTOOL DAC TOOL allows reading and manual setting of the shims or the shim values stored in the computer in the case of a spectrometer with manual shims.

SETUP Read in pulse width and decoupler power calibrations and write to the proper ini files. Use when setting up a new X nucleus

CALIBRATION COMMANDS

H90CAL Calibrate the 90deg pulse width for H1.

F90CAL Calibrate the 90deg pulse width for F19

C90CAL Calibrate the 90deg pulse width for C13.

X90CAL Calibrate the 90deg pulse width for X nucleus meaning O17 to P31.

X90CALBB Calibrate the 90deg pulse width with H1 BB decoupling for X nucleus meaning O17 to P31.

DEC90CAL Calibrate the pulse width of the high power H1 decoupler, H only.

DLPCAL Calibrate the power for the low level H1 BB decoupler, C13 only.

MAIN PARAMETER LIST

SI Set the data SIZE in words; use 8k, 16k etc..

NS Set the NUMBER of SCANS.

RG Set the RECEIVER GAIN. (min=1 ,max=100)

RD Set the RELAXATION DELAY in sec.

N1 NUCLEUS for the first dimension.

F1 FREQUENCY in MHz for the first dimension. See LO.

W1 Set the SPECTRUM WIDTH in Hz for the first dimension.

O1 OFFSET of the center of the full spectrum from the reference for the first dimension.

PW Set the observe PULSE WIDTH in usec.

N2 NUCLEUS for the second dimension.

F2 FREQUENCY in MHz for the second dimension. See DLO.

W2 Set the SPECTRUM WIDTH in Hz for the second dimension.

O2 OFFSET of the center of the full spectrum from the reference for the second dimension.

DLP Set DECOUPLER LOW POWER.(0-50db)

Information Commands

FW Set the FILTER WIDTH in Hz. Default is (w1*1.25)Hz for Gen1 and Gen2 spectrometers and (w1*1.50) for Gen2a.

LO Observe LOCAL OSCILLATOR frequency=(F1+10.70)MHz. This is of interest if the instrument includes a frequency synthesizer under manual control.

DLO DECOUPLER LOCAL OSCILLATOR frequency.=(F2+10.70)MHz

H1 EXPERIMENTS

ZG Zero and Go standard 1D acquisition.

BAPR Acquisition in blocks, allows longer acquisitions with peak registration to compsensate for field drift.

COSY Correlation Spectroscopy helps determine which protons are strongly coupled to one another.

COSY45 Same as COSY but gives smaller diagonal peaks.

INVREC Measure T1 with an Inversion Recovery pulse sequence.
CPMG Carr-Purcell-Meiboom-Gill pulse sequence for measuring T2. Proper use of this pulse program is a major project.
KINETIC Take data at programmed times for measurement of chemical kinetics.

H1 INITIALIZATION FILES
H1.ini Standard H1 acquisition conditions. (default for H1>)
COSY.ini For COSY and COSY45
Prep.ini 40000Hz sweepwidth for locating the field and shimming
Dec.ini For calibrating the DECOUPLER when observing C13.

C13 EXPERIMENTS, Note that H1 BB Decoupling is standard for C13
ZG Zero and Go standard 1D acquisition with H1 BB decoupling.
ZGH Take one scan of the H1 Spectrum using default conditions to for example make sure the H1 spectrum is properly positioned, TMS at 0ppm.
BAPR Acquisition in blocks with H1 BB decoupling, allows longer acquisitions with peak registration to compensate for field drift.
ZGGD Gated Decoupling, decoupling during the relaxation delay but not during data acquisition, gives coupled spectra with NOE.
DEPT Spectral editing: DEPT45, CH, CH2 and CH3 groups give upright signals. DEPT90, CH2, and CH3 signals null, CH signals are upright. DEPT135, CH, and CH3 groups give upright signals, CH2 signals are inverted. The signals from carbons without an attached proton are absent in all three experiments.
HET Hetcor, 2D correlation of H1 and C13 chemical shifts by one bond coupling.
INVREC Measure T1 with H1 BB decoupling.

C13 INITIALIZATION FILES
C13.ini Standard C13 conditions. (default for C13>)
HET.ini For hetcor.

X Nucleus EXPERIMENTS, Note that H1 BB Decoupling is not standard
(See the initialize command for setting up an X nucleus)
ZG Standard acquisition conditions.
ZGBB Standard acquisition conditions with H1 BB decoupling.
ZGH Take one scan of the H1 Spectrum using default conditions to for example make sure the H1 spectrum is properly positioned, TMS at 0ppm.
BAPR Acquisition in blocks, allows longer acquisitions with peak registration to compsensate for field drift.
BAPRBB Acquisition in blocks with H1 BB Decoupling, allows longer acquisitions with peak registration to compsensate for field drift.
INVREC Measure T1.
INVRECBB Measure T1 with H1 BB decoupling.

MAINTENANCE MODULE PROGRAMS
SHIM Shim Y and 2nd order gradient spinning, H2O or non-degassed sample.
SHIM2 Shim all axial gradients spinning, H2O sample.

SHIM3	Shim Y, 2nd and 3rd order gradients spinning, 5% EB sample.
SUPREP	A combined program to synchronize the shims, shim the radial gradients spinning, then the first and 2nd order axial gradients, set the spin rate and finally roughly set the field to the right value. Does a more complete job on the radial gradients than PREP in the H1 folder.
SHUNT	Sets the field offset value and then guides one through the procedure for adjusting the magnet shunt to adjust the coarse field value.
SETSHIM	Set the shim values in the computer to the hardware values. Used for manual shim systems only, Gen1 60MHz.

SYSTEM INITIALIZATION FILES

PNMR.CFG	Indicates to the software, the type of shim (manual or computer), shim debug level, the spectrometer H1 frequency, the IF frequency, tolerance boundaries for shimming, and the Z0 or Offset calibration.
SHIM.CFG	Indicates to the software which shims are present and their adjustment ranges.
SHIMSET.SHM	Contains the shim settings, from the last time a shim program was run or the field offset set.