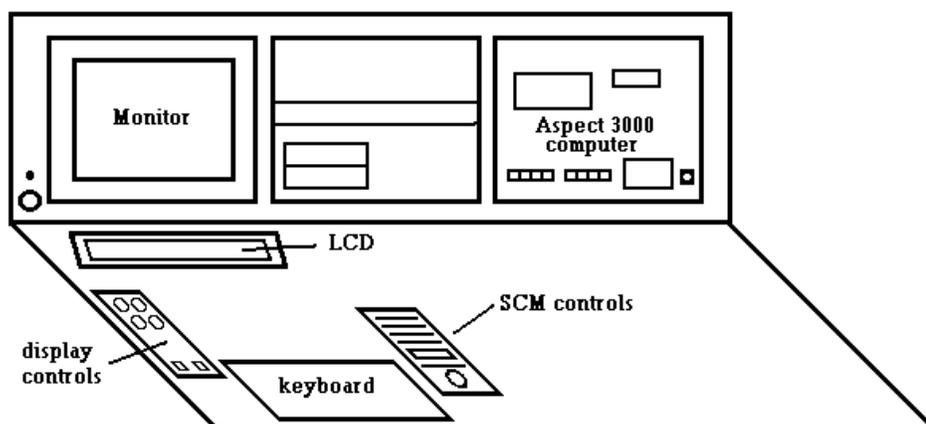


Bruker AM-250 Instructions for Operation

Overview of the AM Console

The Bruker AM Console has a color **monitor** and a **keyboard** from which commands are entered. To the right of the keyboard is the **system control microprocessor (SCM)**, a panel of buttons which operate the shims, the eject and spinner air, and the lock system. Although these functions are digitally controlled, they are operated independently of the main computer. To the left of the keyboard are the **display controls**, consisting of a pair of vertical display buttons and four round **knobs** labelled A, B, C and D. The vertical display buttons, labelled "+" and "-", multiply (+) or divide (-) by 2 the vertical scale of the data displayed on the monitor. The A, B, C and D knobs control a number of aspects of the data display in a manner which depends on the software. Just below the monitor is a **liquid crystal display (LCD)** which normally just repeats all the text input and output which appears on the monitor screen, but sometimes shows messages, errors, and lists which do not appear on the screen. On the right side of the console is the computer (Aspect 3000) which is primitive by modern standards. Indicator lights on the computer tell you when the computer is reading from or writing to the disk, and when data is being acquired.



Monitor. The monitor has a knob at the lower left which turns on the monitor and adjusts the brightness of the display. The monitor displays the lock signal, the computer text input and output, and the actual NMR data, all in contrasting colors. A grid is displayed on the screen which is useful for setting the lock parameters and adjusting the shims - enter *Ctrl-D* to toggle the grid on and off. If you see only the lock signal, or only the data, use *Ctrl-L* to toggle to the correct display. If you see the FID or spectrum divided horizontally into two parts, use *Ctrl-Y* to see only one data display.

Keyboard. Note that this is not a standard keyboard: The equals (=) sign is an upper case key at the upper right of the "p", the colon (:) is a lower case key to the right of the semicolon, and the asterisk (*) is the upper case version of this key. To the left of the orange "control" key is a "print on-off" key which, when activated, sends all text output to the printer. This is useful for printing out parameter lists, peak lists, and automation programs. To execute a control command such as *Ctrl-H* (halt acquisition), you hold down the "control" key while

pushing the "H" key. To the upper left of the control key is an "escape" key and above it is an "alpha lock" key which is the equivalent of the "Caps Lock" key on most keyboards. The alpha lock key is normally depressed, since all input needs to be upper case. Finally, to "backspace" incorrect input from the keyboard, you must use the "Del" key at the bottom right corner of the keyboard. Instead of making one character at a time disappear at the command line, however, the deleted characters are repeated in brackets. So if you enter "BROOKER" and then hit the "Del" key five times followed by "UKER", the command line on the monitor will read "BROOKER[REKOO]UKER". Pressing "Return" at this point will input "BRUKER". When entering the numerical value of a parameter, pressing the "Del" key once will start entry over again with the ">" prompt.

System Control Microprocessor (SCM). There is one round knob at the bottom which can be used to control to a large number of functions. Pressing a button turns that button's light on and displays the current value of that setting on a small display in the center. The knob is then activated to adjust the value of that setting; the previous setting is still displayed on the left side of the numerical display while the current setting is changing on the right side as you turn the knob. Pressing the same button a second time will return the setting to its original value - a very useful function if you are not sure the adjustment did any good. Other buttons (Lift, Lock, Spin) simply turn on various functions. Pressing these buttons a second time toggles the function off. The orange button at the lower right is a "second function" button: pressing it before pressing another button changes the meaning of the button to the function printed below the button rather than on it. For example, pressing the orange button and then the "Lift Off" button actually activates the "Lift" function. Sometimes the SCM displays go blank, indicating a crash of the microprocessor. This happens a lot in dry weather due to static discharge when you touch the metal knob - be sure to touch the grounding mat before touching the SCM controls. Re-starting the SCM must be done by facility personnel.

Logging On

To the left of the plotter is a PC terminal devoted to keeping track of billing for the AM-250. To log in, enter your login name at the keyboard. Make sure you don't have the "Caps Lock" set or all input will be in upper case characters and your login will not be recognized. You will be prompted for your password, and then the spectrometer is enabled (audible "click") and your billing period starts. If you are not logged on, the keyboard will not work on the spectrometer. When you are finished with the spectrometer, be sure to type "EXIT" to log yourself off (another audible "click"). If you don't, the people who come after you will be quite happy to let you pay for their time.

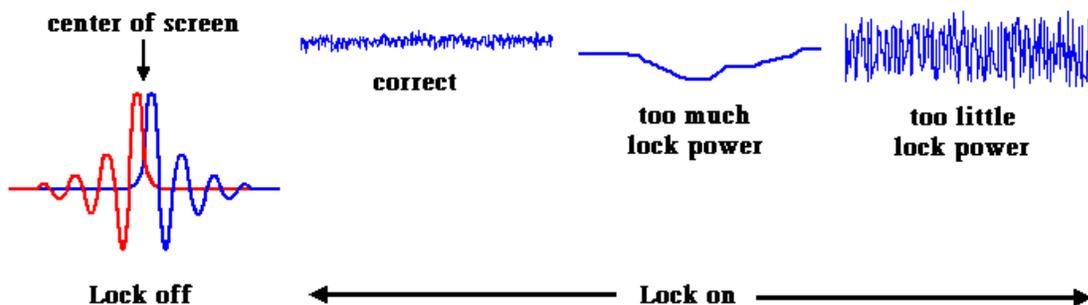
Sample Insertion, Lock, and Shimming

Inserting the Sample. Turn off the spinner air ("Spin") and the lock at the SCM. Eject the sample by pressing the orange "second function" button followed by the "Lift Off" button. Pick up the sample and spinner at the top of the magnet bore, remove the sample from the spinner and position your sample in the spinner using the plastic depth gauge. Clean your tube and the bottom part of the spinner using a KimWipe soaked with ethanol, and set the sample and

spinner on the air cushion at the top of the magnet bore. Press the "Lift Off" button (turns off the lift air; does not put the magnet into orbit) and the sample will be gently lowered into the magnet. When the sample is seated in the probe, press the "Spin" button to start the sample spinning.

Locking. As soon as the sample enters the probe, you should see a deuterium signal on the monitor (assuming that your solvent is deuterated). If you are familiar with the Varian lock system, this will look very different. A line sweeps back and forth across the screen and forms a peak with ringing "wiggles" each time it passes through the deuterium resonance. This is simply a deuterium NMR spectrum of your sample. If you don't see any moving lock line, try **<control> L**, which toggles between three displays: lock and spectrum, lock only, and spectrum only. If you see the lock sweep but no deuterium signal (no "wiggles") the signal is probably outside the sweep window you are observing on the screen. You can move the signal by activating the "Field" button and adjusting the field with the knob. Generally, if you are using the same solvent as the last sample you will see the lock signal on the screen. If not, there is a list of approximate field values for various lock solvents on a card to the right of the SCM controls ("Typical Settings"). Adjust the field to center the lock pattern around the center grid line on the screen. If you don't see a grid, try **<control> D**, which toggles the grid on and off. The tallest peak of the leftward sweep should be equal in height to the tallest peak of the rightward sweep, and the two peaks should be equally disposed horizontally about the center grid line. If the pattern is unsymmetrical (the two peaks are of unequal height), you should adjust the "Lock Phase". With the "Lock Phase" button activated, turning the knob clockwise will lower the right-hand peak and raise the left-hand peak.

When the lock signal is centered and symmetrical, press the "Lock" button to turn on the lock. The sweep signal should become a horizontal line that gradually rises on the screen to a stable level. If the lock doesn't "catch" (remains at the baseline level of the display) you can press the "Field" button and give the knob a slight turn one way or the other (usually clockwise) to "nudge" the lock system into lock. After any of these adjustments it's a good idea to press the "Std By" button to disengage the knob from the previous adjustment. In the "Standby" mode the numerical display shows the spin rate setting and actual value. Sometimes an increase in lock



power or lock gain is necessary to get the system to lock. Both lock power and lock gain will increase the lock signal, but it is important to understand the difference. Lock power is the amount of radio frequency power fed into the deuterium coil in the probe, and lock gain is the

amplification of the lock signal coming out of the probe before it is displayed. Too much lock power can saturate the deuterium nuclei in the sample, leading to an unstable or "breathing" lock signal. It is very difficult to shim using an unstable lock signal. I usually try to have a noise level in the lock signal of about a tenth to a quarter of a grid division; if there is no noise, reduce the lock power and increase the lock gain until the lock level is returned to the next to the top grid line. If there is too much noise, increase the lock power and use the lock gain adjustment to bring the lock level back down to the next to the top grid line. This is a convenient place to put the lock level, since it provides a reference in terms of what is an improvement and what is not when you are shimming.

Shimming. For routine work, you should only have to adjust the Z and Z² shims. For each shim, try to bring the lock signal to a maximum height and then keep going until it goes down again. Then go back until you reach the maximum again. Go back and forth between the Z and Z² shims until you no longer get any improvement in lock level. If the lock level reaches the top of the screen, decrease the lock gain to bring it back to the next to the top grid line. You may want to try to interactively shim Z and Z², since an improvement may require changing both shims at the same time. To do this, move the Z² shim to the right (clockwise) until the lock level goes down about half a grid division. Take note of the direction you moved (right) so you will know which way to go if you get an improvement. Then adjust Z and see if the level can be made better than it was before Z² was changed. If not, return Z to its old setting (this is conveniently done by pushing the Z shim button a second time before selecting another shim). Then move Z² back to its optimum value and adjust it further to the left (counterclockwise) to move the lock level back down by half a grid division. Try Z again to see if a better lock level can be obtained compared to the original optimum level. If not, return both Z and Z² to their original settings. If either of these attempts leads to an improvement, keep moving Z² further and further in the same direction until no further improvement is obtained. Don't be shy when adjusting the shims – some shims require several turns of the knob to see an effect and some are very sensitive. Be sure you have seen a real effect and not just a random fluctuation of the lock level.

Sometimes the shims are hopelessly out of adjustment, and the best strategy is to start over with a "reasonable" set of shim values. These are stored regularly on the disk, and you can recall the most recent set of shims by entering **RSH CURRENT.SHM=D2 <return>** at the job number prompt. Then you can start shimming again from this more reasonable starting point. Since your NMR tube is different (solvent and depth) from others, you will still have to adjust Z and Z² but hopefully the other shims you don't adjust will be better.

Setting Up for Data Acquisition

The Computer. At the bottom of the monitor display you will see some text. The prompt for text input is a number: 1. (or 2. or 3.). This indicates which "Job" you are using. The computer has three jobs: Job 1, Job 2, and Job 3, each with its own data and set of parameters. This is equivalent to the "experiments" in the Varian software: exp1, exp2, etc. To switch from one job to another, just type in the job number: e.g., 3 to move to job 3. This is so simple that sometimes you get confused if you accidentally enter a number and enter a new job. The prompt will always tell you which job you are in, however. In addition to the three jobs, it is possible to

enter the operating system to look at file directories, copy files, print out text files, or delete files. To enter the operating system (called "ADAKOS") simply enter *Cntrl-X* at the keyboard. You will see the prompt "2S:" appear at the liquid crystal display (LCD) above the keyboard. Pressing the "return" key will display an asterisk (*) in the LCD window: this is the ADAKOS prompt. More information about file management using ADAKOS will be given below. ADAKOS can be used while any process is running on the monitor. To return to the monitor and the NMR program ("DISR91") simply enter *Cntrl-X* again. It is important to watch both the monitor and the LCD for responses to every keystroke you enter at the keyboard. **This is not a user-friendly program!** Commands have different meanings depending on which routine of the software you are in (e.g., EP mode, Phase subroutine of EP mode, ADAKOS, etc.) and sometimes must be followed by <return> but at other times the return will be interpreted as a separate command. When you enter a parameter value as a number followed by a unit (**K** for kiloword, **P** for ppm, **H** for Hz, **M** for milliseconds, **U** for microseconds), there is no need for a <return>. This also true for the responses **Y** (yes) and **N** (no): they do not require a <return>. Patience and careful thought about where you are, what you are doing, and how the computer is likely to respond are essential.

Recalling a Set of Standard Parameters. For most routine experiments, the optimal parameters are on disk. To recall a set of parameters, enter **RJ <filename>.<extension>=D1** at the job number prompt. Here are some of the standard parameter sets available:

¹ H spectrum with CHCl ₃ solvent	RJ PROTON.CHL=D1
" with d ₆ -acetone solvent	RJ PROTON.ACT=D1
" with D ₂ O solvent	RJ PROTON.D2O=D1
" with CD ₃ OD solvent	RJ PROTON.MET=D1
" with d ₆ -DMSO solvent	RJ PROTON.DMS=D1
¹³ C spectrum with CHCl ₃ solvent	RJ CARBON.CHL=D1
" with d ₆ -acetone solvent	RJ CARBON.ACT=D1
" with D ₂ O solvent	RJ CARBON.D2O=D1
" with CD ₃ OD solvent	RJ CARBON.MET=D1
" with d ₆ -DMSO solvent	RJ CARBON.DMS=D1
DEPT-45 spectrum	RJ DEPT.001=D1
DEPT-90 spectrum	RJ DEPT.002=D1
DEPT-135 spectrum	RJ DEPT.003=D1

The "=D1" part specifies that the parameters are stored on disk unit 1, which is the location of all system software and standard parameters. Do not delete or save any data or parameters on disk unit 1. If you are using a solvent which is not listed above, choose a parameter set for a solvent with a similar residual ¹H chemical shift. For example, for C₆D₆ (d₆-benzene), which has a residual ¹H shift of 7.16 ppm, use the *.CHL parameter set (CDCl₃ residual ¹H shift is 7.24 ppm). After recalling a set of parameters, you should enter "**II**" (initialize interface) to set all the hardware connections appropriately for the experiment.

Acquisition Parameters. The parameters can be examined by pressing the "Escape" key on the keyboard repeatedly. This moves through a series of four parameter lists (similar to

Varian: dg, dg1, dg2) and then returns to the normal display. The first list shown is the acquisition parameters:

Bruker	(Varian Equivalent)
SF Spectrometer Frequency (in MHz)	
SF0 Exact Spectrometer Frequency used (SF + O1)	sfrq
O1 Transmitter Offset (in Hz)	to
TD Total number of Data Points being acquired (= 2 * Number of Complex Points)	np
SW Spectral Width (Hz) in the Observe Dimension	sw
FW Filter Bandwidth (set automatically from SW)	fb
HZ/PT Digital resolution in the Observe Dimension	
PW Pulse width for simple one-pulse experiment	pw
RD Relaxation delay for simple one-pulse experiment	d1
AQ Acquisition time in seconds	at
NS Number of scans to be acquired	nt
O2 Decoupler Offset (in Hz)	do
SI Size: Number of Data Points after Zero-Filling	fn
DW Dwell Time between Data Points	
DE Delay between observe pulse and acquisition	alpha
RG Receiver Gain	gain
TE Temperature (for Variable Temperature operation)	temp
NE Number of Experiments (Increments) in 2D Acquisition	ni
DS Number of Dummy (Steady-State) Scans	ss

The other parameter lists are not important for routine work. Be sure to distinguish between the letter O ("oh") and the number 0 ("zero"); for example, **O1** and **O2** use the letter O (for "offset"). Also, if you've never used a computer you may still occasionally use the lower-case letter l ("ell") for the number 1 ("one"): you will get in trouble with Bruker! You can also check the value of a parameter by simply typing in its name followed by <return> (unlike Varian <name>? <return>). The present value of the parameter is displayed and the computer waits for input of a new value. If you are happy with the present value, simply press <return>. To change it, enter the new value followed by <return> (unlike Varian: <name>=<value> <return>). If you make a mistake while entering a numerical value, pressing the **Del** key will delete the entire number and give you a prompt (>) to re-enter it from the beginning.

Acquiring Data

Starting the acquisition. There are three ways to initiate acquisition: **GS**, **ZG**, and **AU**.

GS (Go-Setup) is for optimization of parameters: the spectrometer repeatedly scans and displays the FID without summing the individual FIDs to memory. This is an excellent way to shim or adjust the receiver gain, with immediate feedback in the displayed FID. The FID is even integrated to give a single numerical measure of the size of the FID, which can be used as an

alternative to the lock level as a measure of field homogeneity for shimming. In **GS** mode, repeated acquisition of single scans will continue indefinitely and must be stopped with *Cntrl-H*.

ZG (Zero-Go) is for acquisition of a simple 1D spectrum. It executes the sequence **ZE - RD - PW - ADC** where **ZE** zeroes the memory, **RD** is the relaxation delay in seconds, **PW** is the pulse width in microseconds, and **ADC** (analog-to-digital converter) is the acquisition of data from the receiver. The **RD - PW - ADC** sequence is repeated **NS** times, adding the FID to memory each time. **ZG** cannot be used for a ^{13}C acquisition since it will not give any ^1H decoupling – you will see a fully ^1H -coupled ^{13}C spectrum with very low signal-to-noise ratio.

AU (Automation) is for more complicated experiments involving multiple pulses and delays. It must be followed by an automation program name (<filename>.AU) which is a text file spelling out the steps to be followed by the hardware. If no name is specified, it simply executes the last **AU** program that was accessed on the computer. For example, to run a DEPT spectrum simply enter **AU DEPT.AU** after the correct parameters have been set. A simple AU program might look like this:

```
1 ZE      ;zero memory
2 D1      ;relaxation delay
3 P1      ;pulse on transmitter channel
4 GO=2    ;acquire FID
EXIT
```

The first step zeroes the memory so previous data is not added to the new data. The second step directs the spectrometer to wait a period of seconds specified by the parameter **D1** (relaxation delay). The third step executes an RF pulse of duration **P1** in microseconds, and the fourth step acquires the FID and loops back to step 2 for **NS** repeats. For routine work, you will not need to read or understand automation programs.

For proton acquisition, it is convenient to adjust the receiver gain automatically using the command **RGA**. This prompts the spectrometer to try a variety of receiver gain settings until the largest setting is found without overflowing the digitizer (ADC). When the process is complete, the message "AUTO RG FINISHED" is displayed on the LCD and RG is set to the optimum value. You can also use **GS** and adjust the receiver gain interactively while observing the effect on the size of the FID. The left edge of the FID should not extend vertically more than two grid divisions above and below the center. If it does, the data will be truncated ("clipped") because it overflows the analog-to-digital converter (ADC). This clipping will lead to an oscillating baseline in the transformed spectrum. The receiver gain (**RG**) is adjustable in steps (1, 2, 4, 8, ..., 64, 100, 200, 400, ..., 1600) and can be changed while pulsing in **GS** mode. To maximize the dynamic range it should be set as high as possible without exceeding the + or - 2 grid division limit. Use *Cntrl-H* to stop pulsing and exit **GS** mode. For carbon spectra, **RG** will be set to maximum by the standard parameters and usually will not have to be adjusted.

Before starting the actual acquisition, set the number of scans (**NS**). For reasonably concentrated samples, proton spectra require only 16 scans. More scans will be required for more dilute samples, with the number of scans required proportional to the inverse square of the concentration. For example, a sample at 1/3 the concentration will require 9 times as many scans to get the same signal-to-noise ratio. **NS** should be a multiple of 8 to allow for the complete

phase cycle, a process which eliminates artifacts in the spectrum. For carbon spectra **NS** is set to -1 (minus one), which means that repeated scans will continue until stopped with **<control> H**.

Carbon acquisition is usually carried out with a simple AU program called CARBON.AU. This is just a 1-D single-pulse sequence, but the decoupling of protons is switched between two different power levels to limit sample heating.

Processing Data While Acquiring. For ^{13}C acquisitions, you might want to see how much signal-to-noise ratio you have in your spectrum without actually stopping the acquisition. This is done by transferring the current summed FID to another job and processing it while acquisition continues in the present job. Start with the command **TR** (transfer). The computer displays a job number, which is the destination job for the transferred FID. Either accept this value or change it, and the current sum will be transferred to that job. **TR** also moves you to the destination job and displays the FID. Now you can process it just as you would a completed acquisition. You can return to the original job, in which acquisition is continuing, whenever you wish. Don't forget that you still have to halt the acquisition (*Cntrl-H*) before you take out your sample and walk away!

Removing Your Sample and Inserting the Standard

Turn off the *Spin* and the *Lock* and eject your sample using the *Lift* button. Insert the 1% CHCl_3 in CDCl_3 standard (sealed tube) and adjust the lock settings and lock. Be sure to log off by typing **EXIT** at the billing PC.

Rebooting the Aspect 3000. Sometimes the AM-250 computer "hangs" and it is necessary to re-boot. This is not as scary as it sounds, but first be sure there is no acquisition going on and that all data is saved to the disk (**WR** command). On the Aspect 3000 computer, located at the right side of the console front panel (see diagram on first page), there is a row of black buttons. Above them the roman numerals **I**, **II**, and **III** have been written over the buttons for **STOP**, **CLEAR** and **DISK**. Press these three buttons in that order and then on the keyboard hit the return key twice. There will be lots of things going on on the screen and some beeping. When all of this is done, enter **ZE** to clear the data and you may want to reset the plotter pen colors since they are all reset to pen 1 (black) when you reboot. Enter **PEN <return>** and answer the questions with L, 1, 2, 3, 2, 3, etc. for the colors corresponding to pens 1, 2 and 3 (usually black, blue and red).

Processing the Data

Remote Processing. Since January of 2003 we have used a PC to process NMR data from the Bruker AM-250. This allows the use of faster, more powerful and more user-friendly software (Bruker XWinNMR) with a better graphics display, as well as getting the user off the console sooner so that the next user can acquire data. As soon as you finish acquiring your FID, the binary FID data is transferred to the PC. At this point you can remove your sample, insert the standard sample, lock and adjust the lock parameters. The FID will be available on the PC and you can log off the Bruker console and continue processing your spectrum at the PC. XWinNMR is the same software package used for data processing on the Bruker DRX-500 and DRX-600 instruments. First we will discuss processing data on the Bruker AM-250, since you will want to verify that your data is OK (good shimming, sufficient signal-to-noise ratio, etc.) before logging out or moving on to another sample.

Transforming the FID. When acquisition is complete, you can save the raw data (FID) to disk by entering **WR <filename>.<extension>=D2**. **WR** (write) copies the FID data from memory to disk unit 2 along with the parameters. The rules for filenames are the same as for DOS files: 8 characters followed by up to 3 characters extension. If a file already exists by that name, you will get a prompt "**DELETE ON D2? (Y,N,Q)**" on the liquid-crystal display. This can easily be overlooked if your attention is focused on the monitor. Answering "**Y**" will destroy the data in the old file. For convenience in listing, transferring and archiving your NMR data files, it is required that you use the first four characters of your login for all of your files; for example, **SMIT____.____=D2** if your login ID is "smith". After you transfer the file to the PC and convert it to XWinNMR format, you can name it anything you want, with no need to identify your name. If you forget to save the raw data (FID) on the disk, you can get it back at any time with the command **RE FID#**, where # is the job number where the data was acquired. This will bring the original FID back on the screen, and you can save it to the disk with the **WR** command.

Check the parameter **LB** (line broadening) to assure that the correct exponential multiplier will be used. The command **EM** applies this window function. Check that the parameter **AI** (absolute intensity) is set to zero. This feature is used for making quantitative comparisons, but can cause real havoc with your spectrum. The command **FT** performs the Fourier transform and displays the full spectrum on the screen. The two commands **EM** and **FT** can be combined in the single command **EF**. Phasing, referencing, and setting the plot limits are all accomplished in a subroutine called **EP**.

Using the EP Mode for Spectrum Display. Enter the EP subroutine by entering **EP** at the keyboard. To exit EP, simply hit the <return> key. EP is an interactive spectrum display routine similar to the Varian **ds**. Commands are normally two-letter abbreviations outside the EP subroutine, and one-letter abbreviations within EP.

Within EP, the knobs to the left of the keyboard labelled A, B, C, and D are activated. Use the A and B knobs to adjust the **display window**. The A knob moves the displayed portion of the spectrum left or right and the B knob expands or contracts the region displayed. The C knob controls a cursor (white triangle), which rides on top of the spectrum and can be used to indicate peaks or chemical shift positions. You can set the expansion interactively by placing the cursor on the desired left edge of the display window and entering **R**. Use the C knob to move a second cursor, originally on top of the first cursor, to the right and place it on the desired right edge of the display window. Entering **R** again at this point will expand the display to include only the region between the two cursors.

To **phase correct** the spectrum, adjust the display window (A and B knobs) so that only the most upfield peaks are displayed. Enter **P** to enter the phase correction mode. The cursor will jump to the top of the tallest peak displayed and the program will attempt to correct its phase. Now the C knob is activated to adjust the (zero-order) phase of this (pivot) peak. If you run to the end of the C knob adjustment, enter *Cntrl-C* to reverse the sense of the knob. When the upfield peaks are correctly phased, move the display window to show the most downfield peaks in the spectrum (turn A knob clockwise). Now correct the phase of these peaks using only the D knob (first-order phase). If you come to the end of the D knob travel, use *Cntrl-D* to reverse the sense of this knob. When you are happy with the phasing of all peaks, enter **M**

(memory) to apply the phase correction and exit the phase mode. To bail out of phase mode without applying the phase correction, simply hit <return>.

Further data processing includes setting the reference chemical shift, generating peak lists, plotting the spectrum and integration. Since you are only processing the data on the AM-250 to verify the shimming and the signal-to-noise ratio, you will not need to learn these tasks on the instrument. After transferring your raw FID data to the Linux PC “larmor”, you will complete the data processing and plotting using the modern XWinNMR software (see below).

Transferring the raw FID data to the PC for data processing with XWinNMR

When you are finished acquiring all of your NMR data and you have saved the raw FID data to the disk (**WR** command), you can send all of the files to the Linux PC “larmor”. This is done in two steps: all files are sent to an old PC using the Bruker program “**SENDZZ**”, then these files are sent to “larmor” using the internet file transfer protocol (**ftp**).

1. Just to the left of the Bruker AM-250 is an old PC with a monochrome screen. Move to the directory C:\DATA\ZZNET and run the data transfer program GETZZ:

```
cd c:\data\zznet  
getzz
```

You will see a spinning prompt indicating that the PC is waiting to receive data from the Bruker AM-250.

2. Back at the Bruker AM-250 console, at the job prompt enter:

```
PASC SENDZZ           Run the Pascal Program “SENDZZ”
```

You will be prompted for the filename (look at the LCD screen above the keyboard):

```
Filename: <filename>=D2
```

Use the name of the file that you just saved to disk. This file will be sent to the PC. Repeat this step, starting with the **PASC SENDZZ** command, for each file that you wish to send. If you give a filename that doesn’t exist, or otherwise get into trouble, use the command **Ctrl-E** to stop the file transfer program on the Bruker console.

3. At the PC, enter:

<control> C	to halt the data transfer program
cd data	to change to the data directory where the FID is located
ftp larmor	to begin the data transfer to the Linux PC “larmor”
Username:	Enter your login ID for larmor
Password:	Enter your password
ftp> bin	Set to binary mode for data transfer

ftp> lls <user>*.*	List all files which start with first 4 letters <user>
ftp> put <filename>	Send the FID file to larmor (don't specify =D2) (repeat this command for each file you wish to send)
ftp> quit	Exit from the file transfer program "ftp"

4. At the Larmor keyboard, enter your login ID and password. If you don't see a menu bar across the bottom of the screen, click on the small arrow at the extreme lower right of the screen. Click on the red hat icon (lower left) and select System Tools and Terminal to generate a text entry window. To get the proper font and spacing in the text window, click on **Settings / Font / Custom** and select **Fixed (Misc.)** for Font, **Regular** for Font Style and **10** for Size. Click on **Settings / Save Settings** and you will not have to adjust the font again. At the Linux prompt enter:

convert	convert FID data to XWinNMR format and run the modern Bruker NMR software package
----------------	---

The program will first display a list of your ^1H and ^{13}C FIDs currently on Larmor. Then it asks you for the Bruker filename (FID) which you wish to process. Enter this filename (the =D2 is no longer necessary).

Next enter the dataset name you will use in the new software. This can be the same as the Bruker filename, but it's better to use a more descriptive name since you don't need to specify your own name or the type of experiment (^1H or ^{13}C) in the filename. For example, if your Bruker name was SMIT0315.13C=D2, you could simply use the name "**0315**" for the dataset name, or better yet something like "**diester_crude_product_0315**" if you want. Underneath this directory name there will be an experiment number, and we will always use experiment 1 for ^1H and experiment 2 for ^{13}C . Thus if you run both proton and carbon on a sample you should use only one dataset name and both spectra will be stored under that name.

Next enter the solvent you used. This is just for bookkeeping since it will appear in the parameter list on your spectrum. You could enter "**CDCI3**" or "**CD3OD**" or "**DMSO-d6**" or whatever you want. Then you will be asked to specify whether the data is ^1H or ^{13}C data, by entering an "**h**" or a "**c**", respectively. After answering this question the XwinNMR software will start.

The first time you run XWinNMR, you will get a display of the Bruker logo on the screen. Click on **File / Dataset Owners / <username>** to tell XwinNMR where to look for your data. After this first time XWinNMR will always start up with a display of your data, and you won't need to specify the username again. To find your new dataset, click on **File / Open / Data Set Names** and select your dataset name from the menu. If you have both ^1H and ^{13}C spectra in this dataset, you might have to switch experiment numbers (**re 1** or **re 2**) to get to the right dataset. Refer to the general instructions ("Reminder Sheet") for XWinNMR (see below) to complete the processing of your data on **larmor**. To process another dataset, exit XWinNMR by clicking on **File / Exit**, and again enter **convert** at the UNIX prompt. Every time you enter **convert** at the UNIX prompt you are running a data conversion program that converts a Bruker AM-250 FID to the XWinNMR format and then starts the XWinNMR program. If you wish to start XWinNMR without converting any data (for example, if you have already converted the

data in a previous session and you just want to process if further), simply enter **xwinnmr** (all lower case) at the UNIX prompt. This just starts the XWinNMR program.

When you have finished, exit from XWinPlot and XWinNMR and then click the right mouse button on the background (“wallpaper”) of the screen. Select “logout” and then “Logout” to confirm. Your data, both raw (FID) and processed (Spectrum) remain saved in your personal area on the Larmor hard disk.

Archiving Your Bruker NMR Data

Larmor has a built-in CD-ROM read/write device, so you can easily archive all of your NMR data to a CD-ROM. You will have “raw” (Bruker AM format) FID data in your login directory:

/home/<user>

and “converted” (Bruker DRX format) FID data as well as all processed data (spectra) in your XWinNMR data directory:

/opt/xwinnmr/data/<username>/nmr

To copy all of this data to a CD-ROM, follow the instructions below:

1. Insert a blank CD in the CD-R/RW 8X/4X drive of the PC.
2. In your Linux text window, enter **xcdroast** at the Linux prompt.
3. Click on **Create CD / Master Tracks**
4. In the **File/Directory View** window (right side), navigate to the /home/<user> directory, where <user> is your login name. Double-click on the **home** folder to open it, and single-click on the <user> folder to highlight it.
5. Click **Add**, select: **Add with last path component (“/<user>”)**, and click **OK**.
6. In the **File/Directory View** window (right side), navigate to the /opt/xwinnmr/data directory, and single-click on the <user> folder to highlight it.
7. Click **Add**, select: **Add to root directory of CD (“/”)**, and click **OK**.
8. The **Session View** window (left side) should have two entries: => /home/<user> => /<user> and => /opt/xwinnmr/data/<user> => /
9. Click on **Create Session Image** (upper right).
10. Click on **Master and Write on-the-fly** (lower right)
11. Answer **OK** to the question: Ready to Write a Single Session CD? You will see the Track bar graph go from 0 to 100% and the Total bar graph go from 0 to 99% as the data is written to the CD. When finished it will eject the CD from the drive.
12. Insert the CD in the top CD drive (just above the CD / RW drive)
13. Single Click on the **cdrom** icon on the screen. When the /mnt/cdrom window opens, verify that all of your data folders and raw data files are in place on the CD. Close the /mnt/cdrom window (click on the X in the upper right corner of the window).
14. Right click on the **cdrom** icon and drag down to **eject**. The CD will be ejected from the drive. Write your name and the date, and “Bruker AM-250 Data” on the CD.

To delete your NMR data on the PC enter in the Linux text window:

```
cd /opt/xwinnmr/data/<username>/nmr
/bin/rm -r *
cd
/bin/rm ABCD*.*
```

where ABCD represents the first four letters of your login ID, in upper case. This will remove all of your NMR data from your data area on larmor. Be careful!

To copy NMR data from the CD back to Larmor:

1. Insert the CD in the upper CD drive (Read Only CD-ROM).
2. Single Click on the **cdrom** icon on the Desktop.
3. Open the **nmr** folder.
3. Single Click on the **Home** icon in the upper left corner of the desktop.
4. Navigate to the /opt/xwinnmr/data/<user>/nmr folder and open it.
5. Click on the dataset of interest in the “cdrom” window and drag and drop it into the /opt/xwinnmr/data/<user>/nmr folder. Select **Copy Here**. Repeat for as many datasets as you wish to copy.
6. Close the **cdrom** window (click on X in upper right). Right click on the **cdrom** icon and drag down to **eject**. Close the /opt/xwinnmr/data/<user>/nmr window (click on X in upper right).
7. Enter **xwinnmr** in the Linux text window to run the Bruker XWinNMR software.
8. Click on **File / Open / Data Sets by Name** and select the desired dataset.

Alternatively you can use Linux commands to copy the data from the CD to Larmor:

1. Insert the CD in the upper CD drive (Read Only CD-ROM).
2. In the Linux text window, enter:

```
cd /mnt/cdrom
ls
cp -r /mnt/cdrom/<dataset> /opt/xwinnmr/data/<user>/nmr/.
```
3. repeat the last command for as many datasets as desired

Disk Operations on the Bruker Console

Eventually you will need to delete files on the Bruker disk, or look at the disk directory to see what you have. There are two ways to carry out disk operations: from the NMR program (monitor display) and from the ADAKOS operating system (LCD display). The disk system is primitive: there are two partitions, D1 and D2, with D1 devoted to system programs and files, and D2 reserved for data. There is a parameter called **DU** (Disk Unit), which specifies the default disk unit; it should always be set to D2. To read files, execute AU programs, or read parameters from D1, you will need to specify the disk unit by adding **=D1** to the filename. For example, to recall the standard proton parameters for CDCl₃ enter **RJ PROTON.CHL=D1**.

There are no directories on either disk unit, and there is no such thing as permissions and file protection: anyone can destroy anything! To find your files on the disk, you would **NOT** want to enter DIR at the job number prompt, because you would get a list of the entire hard disk that would take many minutes to display on the screen. It's better to use a wild card of some sort: **DIR SMIT*.*=D2**, for example, would list all files which start with **SMIT** on disk unit 2 (the first asterisk stands for the second four characters of the filename, and the second asterisk stands for the extension). If your login name is SMITH, this will be a list of all of your NMR files. The list will be presented on the monitor screen, along with the amount of disk space left and the largest empty sector on the disk. Hitting the <escape> key will exit this display. To get a hardcopy directory printout, activate the printer and enter **DIR** followed by a wild card representation, for example **DIR JONE*.*=D2** to print a list of all files which start with **JONE** (all files owned by user JONES). After the printout is complete, de-activate the printer and press the <escape> key to exit the directory display on the monitor screen.

More specific queries can be made in the ADAKOS operating system, where the single-character wild card "?" is supported. To get a directory printout in ADAKOS, enter *Cntrl-X* and <return> to switch to ADAKOS, then activate the printer and enter **DIR** followed by a wild card representation, for example **DIR JAC?.*=D2** to see all files which start with **JAC**. Then disable the printer and exit ADAKOS with *Cntrl-X*.

To delete a file, enter **DEL <filename.extension>=D2**. You will be prompted at the LCD with DELETE ON D2? (Y, N, Q) and must enter **Y** to actually delete the file. This command works in both the NMR program and in ADAKOS. Remember, don't delete or alter any files on D1! If you transfer your files to the PC each time you use the NMR, you won't need to archive your files on the Bruker AM-250. To delete all of your NMR data files on the AM-250, enter **DEL ABCD*.*=D2** at the job prompt, where ABCD represents the first four characters of your login name. Answer **Y** if you want to delete all the files, or **Q** if you want to be prompted for each one to decide individually whether to delete or not.

Reminder Sheet for Bruker Xwinnmr Software

abbreviations:

L : click left mouse button	M : click middle mouse button
LL : double click left mouse	LH : click and hold left mouse button
MH : click and hold middle mouse button	Menu Buttons in Bold Face
C : red diagonal arrow cursor	V : vertical arrow cursor
> XwinNMR prompt	% UNIX prompt

I. Processing Your Data.

1. Fourier Transform: **>trf** (automatically switches display to spectrum window)
2. Adjust Spectrum Display: **L** on buttons in upper left corner of display: **|<>|** to see full spectrum; ***2, *8, /2, /8** to change vertical scale; **<>** to expand and **><** to compress horizontally;

up and down arrows to move spectrum up and down. Interactive adjustment of vertical scale: **LH** on (vertical position) or (vertical scale) and drag up or down.

3. Manual Phase Correction: **L** on **Phase** to enter phase correction mode, set pivot peak by **L** on **biggest** (to use biggest peak) or **cursor** (to position **V** on upfield peak and **M**). **L** on ***2** or ***8** to exaggerate phase errors, **LH** on **PH0** and drag up/down to correct pivot (dotted vertical line) peak, **LH** on **PH1** and drag up/down to correct "other" peak. **L** on **Return**, **L** on **Save and Return** to exit phase correction mode and apply correction to spectrum.

4. Expand Region of Reference Peak: **L** anywhere on spectrum, move **V** to left edge of desired region, **M** and move second **V** to right edge, **M** and then **L** to get back **C**.

5. Set Reference Chemical Shift: **L** on **Calibrate**, move **V** to top of reference peak and **M**. Enter Reference Shift in ppm (no need to specify units).

6. Baseline Correction (for accurate integration and peak picking): **>abs** to automatically flatten the baseline.

7. Integrate: **L** on **|<>|** to display full spectrum, **L** on ***2** or **/2** to adjust vertical scale, **L** on **Integrate** to enter integration mode. **L** anywhere on spectrum and position **V** at left edge of first integral, **M**, move **V** to right edge and **M**. Repeat **M**, **M** for each integral. To highlight one integral: Position **V** within integral region and **L**. Under *Current* bar **L** on **Calibrate** to enter normalized area of this integral or **Delete** to remove it. Under *All* bar **L** on ***2** or **/2** to adjust height of all integrals on screen. To change color of integrals displayed on screen: **L** on **Display, Options, User Interface, integral** and adjust Red, Green and Blue sliders. **L** on **Apply** and **LL** on upper left corner of *User Interface Settings* window to remove it. **L** on **Return** and **Save as 'intrng' & Return** to exit integration mode and save the integral regions for your spectrum plot.

8. Peak Picking: **>pscal**, select **global**. **L** on **Utilities**, **L** on **Y** and **/** or **YU** as required to get a vertical scale in cm, **L** on **Return**. **L** on **Analysis, Peak Picking** and **Define Region**, set **F1** and **F2** values to desired ppm range and accept defaults for everything else. **L** on **Analysis, Peak Picking** and **Adjust minimum intensity**, move horizontal line up to desired threshold level and **L**. **>psign** and **L** on **pos**, **neg**, or **both** to select type of peaks to be picked. To print out a list, **L** on **Analysis, Peak Picking** and **List peaks on screen** and **L** on **Print**.

II. Plotting Your Spectrum.

Do Not Use the Plot Button in XWIN-NMR!

1. Enter plotting program (XwinPlot): **L** on **Windows** and **Plot editor**

2. Set up Printer (first time only): **L** on **Options** and **Printer Setup**, under *Printer Type* highlight **EPSI** (very important!). For thesis margins, set *Left Offset* and *Bottom Offset* to 3.81

(cm) and *Right Offset* and *Top Offset* to 2.54 (cm). **L** on **File, Save as Default** and then **Apply** and **OK**.

3. Get List Information from Spectrum: **L** on **XWIN-NMR** and **XWIN-NMR Interface**, **L** on **Create Parameter List, Create Peak List** (if you did Peak Picking in XWinNMR, step 8 above), and **Edit Title** (**Do not** select Create Integrals). Enter sample description into Title editor (e.g., "Oxime Methyl Ester in CDCl₃ 25C") and **L** on **Save** and **Quit** (blue). This title will appear above the spectrum any time in the future when you return to look at this data. **L** on **Close**.

4. Put Parameters on Plot: Use picture icons on left. **L** on Parameters icon (list of text) and move **C** to upper left of desired region of plot, **L**. **L** on Mark Objects icon (square of 8 green dots) and **L** on parameter list to highlight it with green squares and dotted lines. **L** on **Attributes** and set *Font Size* (7.00) and *Font Type* (Times Roman). **L** on **File** and **Save as Default** to always use this font. **L** on **Set** and **OK**. **LH** on highlighted parameter list and drag to position in Plot.

5. Put Spectrum on Plot: **L** on Spectrum icon and position "+" cursor at upper left of desired spectrum region. **LH** and drag to the right and downward to expand spectrum into desired space on plot. To expand a spectral region, **L** on **Expand** and hold down the *SHIFT* and *CTRL* keys on keyboard while you position vertical blue line at left edge of desired spectral region and **LH** and drag to right edge.

6. Adjust Spectrum: **L** on Mark Objects icon (square of 8 green dots) and **L** on spectrum to highlight. **L** on **1D/2D Edit** and use buttons in *Edit Display Object* window as you do in XWIN-NMR to adjust vertical scale (*2 and /2), position (**LH** and drag on double arrows) vertical and horizontal expansion (**LH** and drag on double triangles) and **L** on **Show Peaks** and/or **Show Integrals** to display chemical shifts and/or integrals on spectrum. **L** on **Close** to exit this window. If necessary, **LH** and drag on green squares at corners of spectrum to re-size the spectrum and **MH** and drag on spectrum to re-position.

7. Plot Spectrum: **L** on **File** and **Print**, **L** on **Print**. **L** on **File** and **Close**, **L** on **OK** to exit Plot Editor and return to XWIN-NMR.

8. Exit XWIN-NMR and Log Out. Your data is already saved and you can return to it at any time by **L** on **File, Open** and **Data Sets by Name** and selecting your dataset by **L** on the sample description (Name). If there is more than one experiment for this sample (e.g., ¹H, ¹³C, APT...) you can select experiments within a sample name by **>re #** where "#" is the experiment number (1, 2, 3...). **L** on **File** and **Exit**, **L** on **OK** to leave XWIN-NMR. Click on the Linux Main Menu icon (Red Hat) and select **Logout**. Click on **Logout** to confirm.