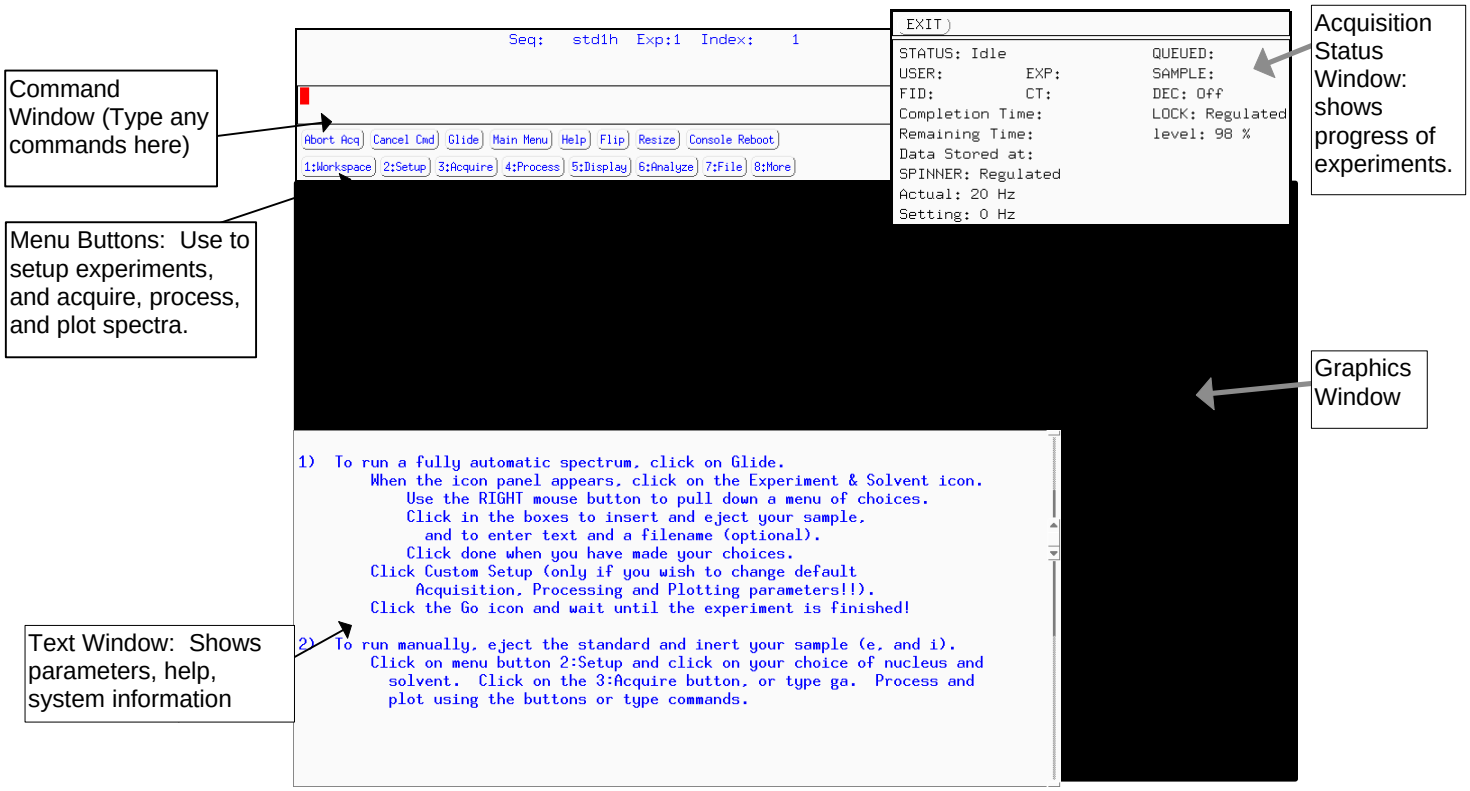
I. GETTING STARTED (LOGGING IN, LOGGING OUT, USING THE NMR PROGRAM).

A. Logging into the Gemini

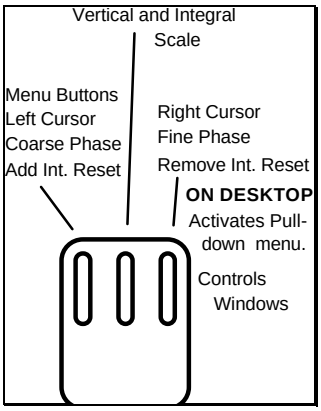
To login onto the Sun workstations, hit the return key. The screen will light up and you should see the login prompt. Type **gemini** and hit return. Then enter the passwd shown by the graphics display and press return once more. If you make a mistake, press return and wait until you see the '**gemini300.la.asu.edu console login:**' prompt once more and try again. Once you have logged in, you will be asked for your last name (or a classname). The Unix graphics window environment will then start (ca. 15 seconds), as will the Varian NMR program, VNMR. When ready for use, the display will look like this:



B. Leaving the Gemini.

When you are finished, you should sign the user logs and exit to make the workstation available for use by others. When you leave the system, you need to exit the NMR program and then exit the window environment. Type **exit** in the NMR command window. The NMR windows will disappear. Next, place the mouse arrow anywhere on the background. Click and release the **RIGHT** mouse button. A pop-up menu will appear. Click on the word **EXIT**. You will be asked to confirm your choice. Click on **EXIT.** After a few seconds, the graphics environment will stop and you will be logged off the Unix workstation..

C. Interacting with VNMR: Tips on Using the Mouse, Entering Commands and Using Menus.



You interact with VNMR by typing commands, by pressing the function keys or the LEFT mouse button to activate menu buttons and icon buttons, and by using all three mouse buttons to manipulate spectral displays.

Mouse buttons: The LEFT mouse button selects choices on menu boxes (you can also press the corresponding function keys F1-F8). You activate a menu function by placing the arrow within the menu box and pushing the LEFT mouse button once. The RIGHT activates any pop-up menus, if present (e.g, Glide and the UNIX wallpaper have pop-up menus).

The three mouse buttons are also used to manipulate spectra in the spectral display window. When a 1D spectrum is displayed interactively, the LEFT button controls the position of the left cursor. Pushing the RIGHT button will display the second or right cursor. The MIDDLE button controls the vertical scale (height) of the spectrum when no integral is displayed. If an integral is present, the MIDDLE button control the height of the integral. When you are interactively phasing the spectrum, the LEFT button is used to make *coarse* phase corrections and the RIGHT button makes *fine* phase corrections.

Entering commands: Commands are typed in the window, and the command will be performed when you press the return key. More than one command can be typed on a line; leave a space between commands. Commands are always lower case. **Commands will not be carried out until you push the RETURN key.**

II. INSERTING, EJECTING SAMPLES.

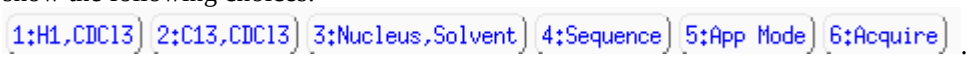
You must first eject the standard sample ($CDCl_3$) and then place your sample in the magnet to acquire a spectrum. Please, use Wilmad 528-PP 5 mm NMR tubes (other tubes will damage the probe). **Please take your time and handle tubes with care. NMR tubes are extremely fragile. Broken tubes and spilled samples may cause expensive damage to the Gemini.**

Eject Sample	**e**	Type **e** to eject the CDCL3 sample.
Insert Sample	**i**	Place your sample in the spinner and use the depth gauge to position the nmr tube. Make certain your sample column =>5cm. Carefully wipe the spinner and clean the nmr tube. Reposition if needed. Put the spinner in the magnet bore, making certain that the spinner is freely floating on the air column, then type **i**. Follow the same steps to remove your sample when you are finished. Leave the sealed CDCL3 sample in the magnet and be sure to sign both the log and the cards before you go.

III. MANUAL ACQUISITION, PROCESSING, DISPLAY AND PLOTTING

A. Setup For Manual Acquisiton.

In order to manually operate the Gemini, you must first setup the hardware for proton or carbon operation and optimized the acquisition window to the solvent of your choice.. Setup is most easily accomplished using the menus. Click on **2:Setup** on the main menu, The setup palatte will show the following choices:



. You can setup for a proton or carbon experiment optimized for $CDCl_3$ by choosing buttons 1 or 2. If you want to choose a different solvent and nucleus, click on **3:Nucleus,Solvent** This will display a choice of nuclei:

.

Choose **1:H1** or **2:C13.** You will then be given a choice of solvents:

Choose a solvent. After the **2:Setup** command is finished, the acquisition parameters will be displayed. Each parameter has a reasonable default value, but you may change any parameter at this point, as desired. For example, if you wish to increase the number of scans from 16 to 64 to improve signal to noise, type **nt=64**. Single quotes are required around values for string parameters (I agree, this is stupid!), so type commands as shown.

Prepare Gemini	**setup('H1','CDCl3')**	Sets spectrometer for ^{1}H acquisition in CDCL3. The command is somewhat cryptic (quotes are required) and is most easily accomplished using the menus.
Automatic lock	**alock='s' or 'n'**	If '**s**' (default), the spectrometer will autolock whenever a new sample is inserted. If you are repeating acquisition with different parameters on the same sample, you can save time by setting **alock='n'** before the second and subsequent acquisitions. **N.B.: alock** must equal **'s'** for the first acquisition.
Automatic shim	**wshim='s' or 'n'**	If '**s**' (default), the spectrometer will autoshim whenever acquisition on a new sample is started. Save time on subsequent acquisitions on the same sample by setting **wshim='n'**. (See **alock**, above).
Turn auto lock and auto shim off	**no**	**no** sets **alock='n'** and **wshim='n'**. **no** saves time if you need to reacquire a spectrum from a sample that is already in the magnet **and** the spectrometer has already locked and shimmed on the sample.
# of transients	**nt=n**	n=desired value, but should be a multiple of 8 to suppress artifacts.
Block Size	**bs=n**	The spectrum can be transformed by typing **wft** after any multiple of n scans is acquired. If **bs='n'**, acquisition must halt before you can observe the transformed spectrum.
Display Param.	**dg**	**dg** displays the primary acquisition parameters.
Number of acquired data points in FID	**np=n**	**np** determines the number of data points that will be acquired in each transient or scan. Because the data are acquired with quadrature detection, the displayed FID contains ½ **np** points.

Number of fourier transformed points in displayed spectrum	**fn=n**	**fn** determines the number of data points that will be FT'd to give the spectrum. If **fn**>**np**, the FID will be zero-filled before transformation. The displayed spectrum will contain ½**fn** points. **fn** must equal 2^n points (i.e., 2048,4096,8192, etc.)
Pulse Width	**pw=n**	**pw** is the transmitter time, in µsec. (The 90° pulse time, **pw90**, can be examined by typing **pw90?**). Typically, a 20° to 30° pulse angle is appropriate for most ^{13}C spectra, while 45° to 90° pulse can be used for ^{1}H acquisitions.
Sweep Width	**sw=n**	**sw** determines the acquisition spectral width, in Hz. For ^{1}H spectra, the default **sw**=4551 Hz (or 4551/300 or 15.2 PPM).
Add comments	**text('abcd..')**	Adds commentary, 176 characters max. Separate new lines with \, enclose text in single quotes (').
Optimize Pulse Width	**ernst(n)**	**ernst(n)** will calculate the pulse width **pw** that gives the best sensitivity for a nucleus with a spin-lattice relaxation time (T_1) of **n** seconds. Typical T_1 values for protons are 0.5-5 seconds. The T_1 values for ^{13}C can be quite long: values of 20-40 seconds are not unusual for non-protonated carbons in small molecules. For ^{1}H, a reasonable guess for **n** is 5 seconds. For ^{13}C, try **ernst(15).**

The minimum command required to prepare the Gemini for acquisition is **setup('nucleus','solvent')** The default values for all other parameters will be automatically set so that a spectrum will be acquired. Make changes in the parameters shown above only if you need to optimize the NMR experiment for your particular sample.

B. Lock And Shim

By default, the spectrometer will automatically lock on the deuterium NMR signal in your sample and optimize the homogeneity of the magnetic field (shim) at the beginning of each experiment. Please see me if you wish to learn how to lock and shim manually.

C. Acquire and Process Spectra

Start the acquisition of a spectrum by typing **ga**. **ga** will cause the instrument to lock and shim on the sample and then acquire **nt** transients (or sample scans). Once **nt** scans have been acquired, the spectrum will be fourier transformed. If **bs** (block size) is used (e.g., multiple of 16), then you can transform the spectrum whenever the number of completed scans **ct** is a multiple of **bs**. Sensitivity of weak samples can be improved by using some line broadening before fourier transformation: try setting **lb=0.1** (Hz) upto 1 for ^{1}H spectra and 1 Hz to 5 Hz for ^{13}C spectra.

The Gemini will automatically carry out wft when acquisition is complete. After **wft** is complete, the spectra can be automatically phased using the **aph** command (any minor phase error can then be corrected using the **6:Phase** button in display mode). The **region** command will also calculate, *but not display*, integrals that correspond to the major groups of peaks in the spectrum.

Acquire fid	**ga**	Acquire, transform and display.
Line broadening	**lb=n**	Line broadening in Hz, as desired.

Window and ft	**wft**	Exponential multiply and fourier transform the FID. **wft** may be performed at the end of each completed block size in order to see the spectrum.
Autophase	**aph**	Automatically phase spectrum.
Calc integrals	**hregions**	Automatically generates a set of integral regions.
Stop acquisition	**aa**	**aa** will abort the acquisiton at the end of the current scan.

D. Display and Manipulation Spectra

The spectrum will be displayed after fourier transformation using **wft** or at the end of an acquisition. The spectrum can be redisplayed at any time after acquisition and fourier transformation by typing **ds**, for **d**isplay **s**pectrum.

Display spectrum	**ds**	Will occur automatically after acquisition on **wft**.

The command **ds** will activate the interactive display and the **1D Display Menu.** You can also display the fid by clicking on the **3:Display** button on the **main menu** and then clicking **1:Interactive.** Study the help text for this menu by clicking the **HELP** button.

In **ds** mode, the interactive display menu described on page 4 (Section III.D) will appear::

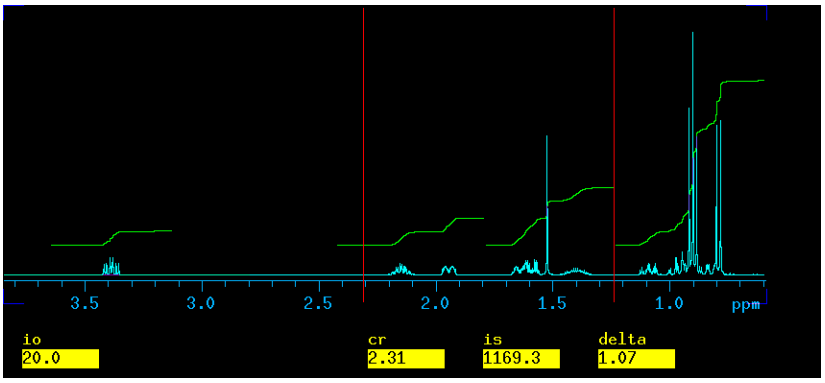
1:Box | 2:Integral | 3:Full | 4:Th | 5:resets | 6:Phase | 7:Next | 8:Return

The Mouse buttons carry out the following actions in interactive **ds** mode: The meaning of each button is shown in the display.

Left The left button moves the left cursor, or the pair of cursors (**1:Box** or **1:Cursor**). It changes the position of the threshold for peak picking whenever a threshold is displayed (**4:th**). If an integral is displayed, it adds an integral reset at the current mouse position. If you press **5:phase**, this button is used for *coarse* phase adjustment. When the mouse is placed at the extreme left edge of the spectrum, the left mouse button is used to adjust the vertical position **vp** of the spectrum or integral offset **io** on the page.

Center The center button changes the vertical scale **vs** of spectrum. If the integral is displayed, it changes the integral scale **is**..

Right The right button positions the second (right) cursor relative to the first (left) cursor. In the **reset** mode, this button removes an integral reset. In the **phase** mode, this button is used for fine adjustment of phase parameters and is less sensitive than the left mouse button by a factor of 8.



Middle button at left edge

Left Middle Right

Expanding a region. In display mode, clock or hold the left or right buttons down to move the respective cursors. The cursors will track the position of the mouse arrow. Place the two cursors around a region of the spectrum. Click **3:Expand** to expand the region between the cursors. Click **3:Full** to display the full spectrum. Click menu button **1:Box** or **1:Curson** to switch between one and two cursors.. Place the mouse arrow above a peak and click the MIDDLE mouse button to increase the vertical scale **vs** of the spectrum to the height of the arrow. The vertical scale can be reduced by a factor of two by placing the arrow anywhere below the baseline of the spectrum and pushing the middle mouse button.

Integration. Click menu button **2:Integral** and an integral is displayed. Type **region** to break up the integral. To add or remove reset points to the integral, click **5:Resets.** The left button will add a reset (break point), the right removes resets. (Note: Each integral requires a start and a stop reset point, so add or remove points in pairs.) When the integral is displayed, the middle mouse button now controls the height of the integrals.

Phasing a spectrum: To phase a spectrum in display mode, display the full spectrum by clicking on the **full** button or type **f.** Carry out an automatic phase adjustment by typing **aph.** You can then make small, manual corrections by clicking on **6:Phase**. Now center the mouse arrow on a large peak at the **right** side of the spectrum, with the arrow halfway up the screen. Click **and hold** the **RIGHT** button to make a fine adjustment or the phase. Next, place the mouse arrow on a peak at the **left** side of the spectrum and adjust its phase using the **RIGHT** mouse button. Use the **LEFT** mouse button **ONLY** if you need to make large phase corrections. When you are finished, click on any of the menu buttons or type **ds.**

Referencing a peak: To reference a peak in **ds** mode, place the left (or single) cursor on the reference peak. Type **nl** (**n**earerst **l**ine). The program will put the cursor exactly on top of this peak. Then type **rl(0)**, if the peak is tms. If you have tms in the sample, you can also type **tmsref** to automatically reference tms to 0ppm. The value in parenthesis is the shift of the peak in hertz and the nmr program only accepts values in hertz. To assign a value in ppm, simply type **p** after the value. For example, for chloroform you would type **rl(7.25p).**

Controlling peak-picking: Click on **4:Th** to display the minimum threshold used for peak-picking. Adjust this line with the left mouse button.

Much of the manipulation of a spectrum can be rapidly carried out using the **Massage** menu. Try the following. Click **Main Menu, 5:Display** and then **2:Massage.** The massage menu shown below will appear.

Click **1:Autophase** to phase the spectrum. Click **2:Region** to calculate integrate regions. You can carry out a spline baseline correction **(3:BC)** or a linear DC offset correction **(4:DC)**. Use **5:Adj VS** to adjust the vertical scale of the spectrum and **6:Adj IS** to adjust the integral scale. Finally, click on **7:Adj WP** to display only that region of the spectrum that has any peaks. Note that you can only adjust the width of the plot AFTER you have calculated integrals. Now click **8:Return**, to get back to the display and processing menu.

The display of the spectrum is controlled by the parameters shown in the following table. The parameters can be entered directly from the keyboard for precise control of the presentation of spectra on the page.

vertical scale	**vs=*n***	Controls height of spectrum (***n*** is in mm and is equal to the height of the tallest peak in the spectrum).
integral scale	**is=*n***	Controls the height of the integral.

start of plot	**sp=*n*p**	Start (right edge) of the region of the spectrum to be displayed. The value of **sp** is in Hz if not followed by **p.** **sp** is in ppm if the value **n** is followed by **p**, e.g. **sp=2** sets the right edge of the displayed spectrum to 2 Hz. **sp=2p** seths the right edge to 2ppm.
width of plot	**wp=*n*p**	Width of the region of the spectrum to be displayed, in Hz. Add a **p** to enter the value of **wp** in ppm.
display the full spectrum	**f**	**f** will set **sp** and **wp** to display the complete spectrum, i.e., all the acquired data is shown after typing **f.**
use the full page for display	**full**	**full** will display the spectra using the maximum physical size on the page. **full** controls the parameters **sc** and **wc**, but does not change **sp** or **wp.**
peak picking threshold	**th=*n***	**th** controls the threshold for peak picking (**thresh**) on alternate **ds** menu.

Many other commands are available for controlling the display of spectra. Several of the most useful are described below: Many of these commands can be executed from alternate menus.

display a scale	**dscale**	Dsplays a scale on the spectrum. The physical position of the scale can be controlled by typing **dscale(*n*)**, where ***n*** is the vertical position of the scale from the bottom of the page, in mm.
display peak frequencies	**dpf**	**dpf** displays the frequencies of peaks on the spectrum. The level at which peaks are picked is controlled by the parameter **th.**
display values of integral regions	**dpir**	**dpir** will display the numeric values for each of the integral pieces (or regions) on the screen. **vp** must be 12 or greater for **dpir** to work.
axis (ppm or Hz)	**axis='p'** or **axis='h'**	If **axis='p'**, the scales and peaks will be shown or plotted in ppm. If **axis='h'** then scales and peaks will be output in Hz.
integral normalization	**ins=*n***	**ins** controls the values of calculated integrals and can be adjusted to calibrate integrals to the area of a known peak.
save display parameters	**s1, s2, to s9**	**s1** saves the parameters that control the display (**vs, is, vp, sp, wp, sc, wc, io, th,** etc.) of spectra in a temporary file. You can save up to nine different sets of display parameters. Display parameters can be moved from one experiment to another using the **md** command described earlier. *NOTE:* **s1** *does not save the actual data, but only the parameters that control the DISPLAY of data!*
recall display parameters	**r1, r2, to r9**	**r1** will recall a display parameter set previously saved using the **s1** command. **s1** and **r1** are useful for returning to an original display after an **inset** plot and provide a means of plotting different spectra in different experiments in precisely the same fashion.
display spectra stacked horizontally	**dssh**	**dssh** will automatically display an array of spectra acquired in the same experiment as a function of some variable, e.g. temperature, T1, noe, decoupling, etc. **dssh** will stack the spectra horizontally across the screen automatically.
display spectra stacked automatically	**dssa**	**dssa** will automatically display arrayed spectra vertically on the screen.

E. PLOTTING SPECTRA

The UNIX computers are networked to both HP7550A pen plotters and to the HP LaserJet4 laser printer. The LaserJet4 is the default plotter. Examine and change the destination (HP plotter or laser printer) for your plots using the following commands:

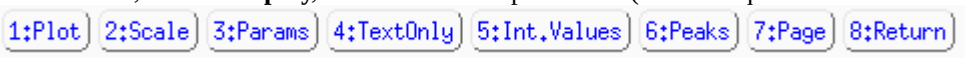
examine plotter destination	**plotter?**	Type **plotter?** and the current plotter device will be shown. The laser printer is shown as **LaserJet_600R** and the HP pen plotter is shown as **HP7550A**
select laser plots	**laser**	**laser** will set the destination for plottting to the HP Laser Jet II. Plots will be output at 600 dots/inch and will be rotated to landscape (sideways) format on the page.
select pen plots	**hp**	**hp** will set the destination for plotting to the HP7550A pen plotter. Plots will come out on 11" x 17" paper, using black, red, green and blue pens.

Once the plotter has been selected, display the spectrum as desired. Plot the spectrum using the commands in the following table. Several commands may be combined on one line in any order, i.e. **pl pir pscale ppf pap page** is perfectly valid. More than one plot can be placed on a page. Simply type **page** only after you have issued all the plot commands that you want on a page.

:

plot the spectrum	**pl**	**pl** will plot the spectrum. The integral will also be plotted, but only if it is displayed at the time the **pl** command is issued.
plot integrals	**pir**	plot numeric values for the integral regions. **pir** will only work if the integrals are displayed.
plot a scale	**pscale**	plot a scale under the spectrum.
plot peak frequencies	**ppf**	plot a list of peaks on spectrum (works best on expansions when trying to pick small peaks in the presence of noise)
plot parameters	**pap**	plot all spectrometer parameters in command format.
plot text only	**pltext**	**pltext** will plot the current text and not the parameters
Inset plots	**inset**	Place two cursors around a region and type **inset**. Plot the inset spectrum using **pl**, **pscale**, **pir**, **ppf**, etc.
finish plotting, eject the page	**page**	**page** will output all plot information (pl, pscale, pir, etc.) and any inset plots to the destination plotter and eject the page.

Plotting can be quickly performed from menus as well. When you have displayed the spectrum as desired, try clicking **Main Menu**, then **3:Display**, and **6:Plot.** The plot menu (and the equivalent commands) are shown below:



(pl) (pscale) (pap) (pltext) (pir) (ppf) (page)

Click **1:Plot** to plot the spectrum and integral (if displayed), **2:Scale** to add a scale to the plot, **3:Params** to plot a table of parameters, **4:TextOnly** if you want to output your sample description without parameters, **5:Int.Values** to plot numeric values under each integral, and **6:Peaks** to label peak frequencies above each peak. Finally, click **7:Page** to output all the information to the plotter or laser printer.

.Spectra will always be plotted exactly as they are displayed. Therefore, the parameters that influence the width, start, and size of the display (shown below) will determine how the spectra will be plotted. Change these parameters to meet your individual requirements.

Vertical Position	**vp=n**	Controls the vertical position of the baseline of the spectrum, in mm. **vp=0** will put the spectrum at the bottom of the page. **PLEASE NOTE: vp** must be 12 or greater in order to print the value of integrals on the plot
Integral Offset	**io=*n***	controls the distance between the baseline of the spectrum and the baseline of the integrals, in mm.
Start of Chart	**sc=n**	Determines the starting (right) edge of the physical chart ("starting position on paper') in mm.
Width of Chart	**wc=n**	Determines the width of the physical chart in mm.

Inset (Overlay) Plots Use the following procedure to create inset (overlay) displays and plots. First, type **s1** to save the current display parameters. You can then return to the original display at any time by typing **r1**. Bracket a region of the spectrum with cursors. Type **inset**. A copy of the region will be placed above the spectrum. The RIGHT mouse button will control the position of the right side of the spectrum (**sc**). The LEFT mouse button will control the physical width (**wc**). The MIDDLE mouse button will control both the vertical position **vp** and the vertical scale **vs.** If the integral was displayed when you typed inset, the MIDDLE mouse button will control the integral scale **is**, instead of the **vs.** Use the mouse to place the inset spectrum to the desired location. You can type **s2** if you wish to recall this spectrum at a later time **NOTE: subsequent plot and display commands apply only to the inset spectrum and not the original display!**

F. PRINTING PARAMETERS, LINE LISTS AND INTEGRALS LISTS

You can capture and print any text that appears in the text window using the **printon** and **printoff** commands. For example, to print the parameter tables associated with the experiment, type **printon dg printoff.** To print lists of peaks and integrals. The following commands can be used to display parameters, peak lists and integral regions in the text window and divert the output to the laser printer, if desired.

display parameters	**dg, dgs, dg1**	**dg** displays the main parameter table. Use **dg1** to display a table of reference and plotting parameters. Use **dgs** to display the value of shims used to acquire the spectrum
display a list of integrals	**dli**	**dli** will display a detailed list of integral regions in the text window.
display a list of peaks	**dll**	**dll** will display a detailed list of peaks in the text window
capture text for output to printer	**printon**	**printon** will capture output to the text window and send it to the printer. For example, to get a list of peaks and integrals, display the spectrum with **ds**, type **printon** and then type **dll** and **dli**. Output the list by typing **printoff**
finish sending text to the printer	**printoff**	**printoff** will turn off capture of text for output to the printer. **You must type printoff to print the parameters and line lists!**

IV. SAVING and RETRIEVING DATA

You have been assigned a directory or folder on the disk for saving your files. You can retrieve spectra at any time from any of the workstations in the NMR facility. Spectra are always saved untransformed (as fid's). Click **Main** Menu, then **7:** files, or type **svf** to save the fid in the current experiment. You will then be asked for a filename. You can use numbers, letters, periods and

underscores (_) in filenames, but DO NOT use spaces. Retrieve a fid by typing **files** or clicking **7:Files** on the **Main menu.** Click on the file you wish to load into memory for processing and click **7:load.** Type **wft** to transform and display the spectrum.

Spectra will be saved for three months. Spectra more than three months old will be automatically deleted by the system at 4 a.m. on Sunday mornings. Spectra can be easily saved to tape. Please see me for instructions.

save the fid	**svf**	**svf** will save the fid to the current disk folder or directory. You will be asked for a filename. Filenames can be upto 44 characters long and can be a mix of numbers, letters, underlines and periods. **Do not use spaces** when making up filenames.
show existing fid files	**files**	**files** will display the list of fids in your current directory. To load a file for processing, click on the file, then click **7:load**