Scheduling

400 MHz A with 100 slot sample changer:
    a. Monday to Friday from 8 am to 8 pm: 10 minute blocks, 30min max.
    b. Monday to Friday from 8 pm to 8 am: 10 minute blocks, unlimited time.
    c. Saturday and Sunday: 10 minute time blocks and unlimited time.

400 MHz B with 12 slot sample changer:
    a. Monday to Friday from 9 am to 9 pm: 10 minute blocks, up to a maximum of 5h per user per day.
    b. Monday to Friday from 9 pm to 9 am: 10 minute blocks, unlimited time.
    c. Saturday and Sunday: 10 minute time blocks and unlimited time.

600 MHz (http://scheduler.yale.edu):
    a. Currently, you may reserve unlimited time in 30-minute blocks, with no maximum. This is likely to change as use increases.
    b. Remember to alert us if you have a time sensitive sample (e.g. degradation issues) or, if you are not able to reserve time (all booked).

Other Issues

Please email WCAC staff if you have issues with the NMRs and leave a note on the whiteboard in the NMR instrument room for other users if you find the instrument not functioning properly.

Please make use of the NMR logbook provided to write down any major or persistent issues.

Feel free to walk into my office and discuss any issues you are having with the instruments and any questions and suggestions you may have. Terence and I are always happy to sit down and troubleshoot with you.
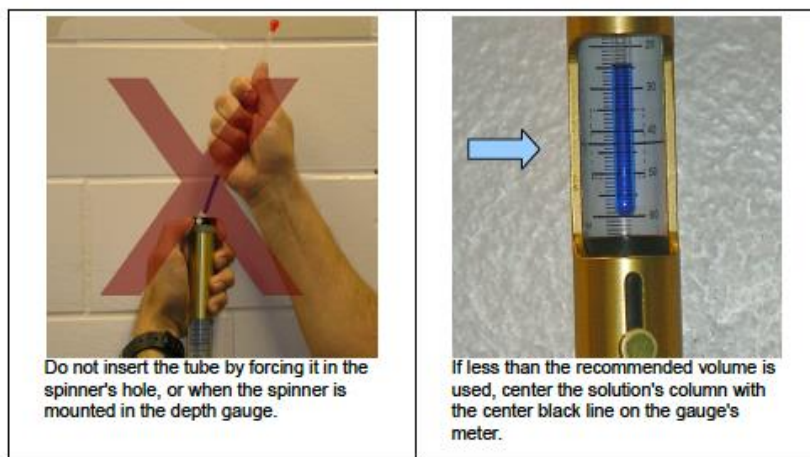
For periodic updates regarding the WCAC including NMR services please visit wcac.yale.edu. You may also signup for our weekly newsletter (West Campus Analytical Core Updates) at https://messages.yale.edu/Subscribe/.

Getting Your Sample in the Magnet

1) Insert your sample tube into a spinner using the sample depth gauge. Gently twist the tube if the fit is tight. If a gauge is not available the bottom of the tube should be 68mm below the bottom of the spinner with the recommended sample volume of 0.7 ml or higher. If you have less than the recommended sample volume center the sample around the coil location shown on the depth gauge.

Do not hold or touch the spinner on the shaft


Do not hold the spinner from the NMR tube.
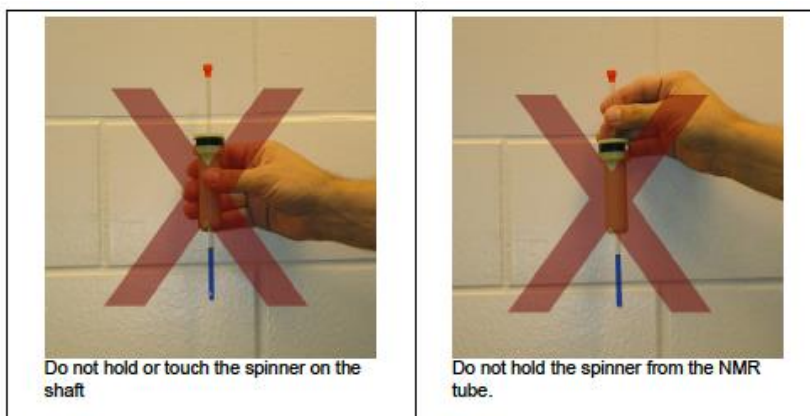
2) Clean the bottom of the tube and the spinner turbine with Kimwipes and ethanol or propanol to remove any residues such as body oils and aluminum oxide from the magnet bore tube.

Do not insert the tube by forcing it in the spinner's hole, or when the spinner is mounted in the depth gauge.

If less than the recommended volume is used, center the solution's column with the center black line on the gauge's meter.

3) Log on to your account on the spectrometer. After VnmrJ has autostarted and displays a green "Idle" status, use the "e" command or eject button to eject the standard sample. Carefully remove the standard from the top of the magnet, place it in the sample holder and place your sample in the top of the magnet. Do not release the sample until you are sure there is a cushion of air to support it. Go back to the spectrometer workstation and enter the "I" command or use the "insert" button. After lowering a sample into the magnet you should hear an audible "clunk" when the sample is in place. If you cannot for example get a lock signal the sample might be stuck in the bore. If this happens try to eject and insert it again. Most broken sample tubes occur when removing tubes from the magnet. Be careful to lift sample tubes clear of the bore tube before moving them away from the magnet.
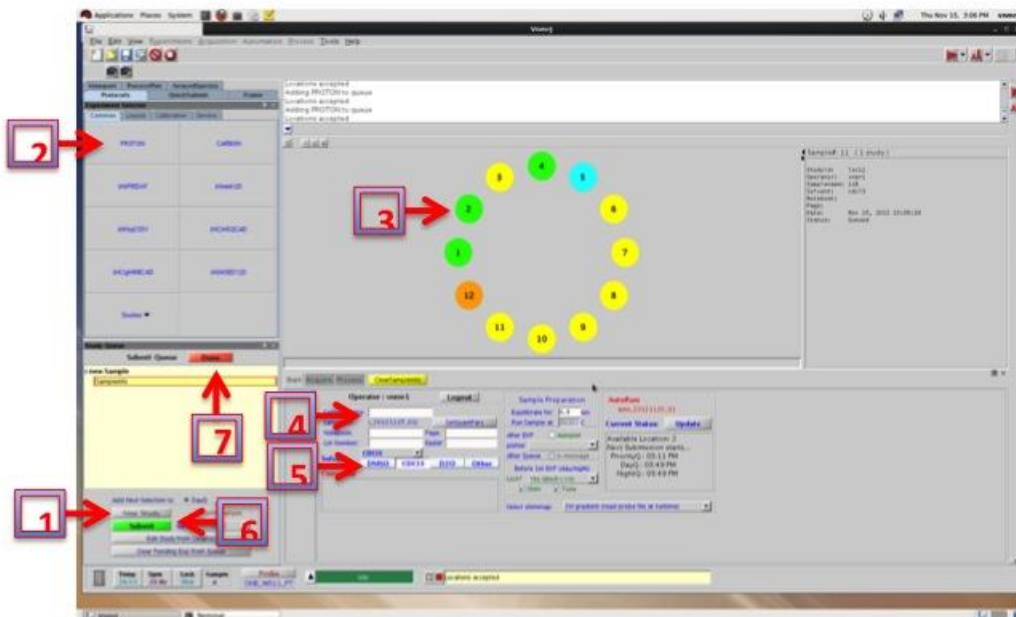
Operation of 400A and 400B
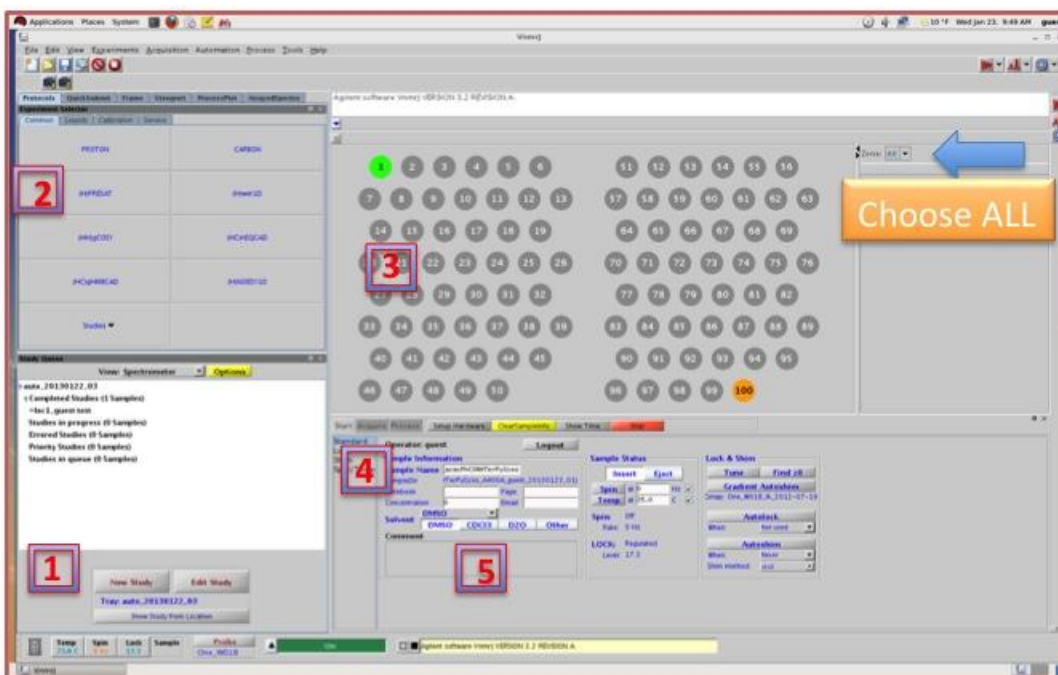
400A: Login as operator Under "guest"

1. Go to Protocols Tab.
2. Click New Study
3. Pick Experiment: e.g. Proton
4. Choose location from GRAY circles: *remember where you placed your sample in AS*
5. Enter Sample Name
6. Choose Solvent
7. Hit Submit
8. Hit Done, start over for next available slot.
9. Logout Operator
10. Exit VNMRJ.
11. Do not Logout "guest" Account.

SAMPLES IN GREY SLOTS 1-99 ONLY.

NO SAMPLE IN # 100 and 99

400B: Login as operator Under 'walkup'

1. Go to Protocols Tab
2. Click New Study
3. Pick Experiment: e.g. Proton
4. Choose location from green circles: *remember where you placed your sample in AS*
5. Enter Sample Name
6. Choose Solvent
7. Hit Submit
8. Hit Done, start over for next available slot.
9. Logout Operator
10. Exit VNMRJ.
11. Do not Logout Guest Account.

SAMPLES IN GREY SLOTS 1-10 ONLY.

NO SAMPLE IN # 12 and 11

Do's and Don'ts

Do:

1. On the **400A ,** check for the <u>next available slot</u> in the software before inserting your sample. The next open spot on the tray is not necessarily the next available spot.
2. Wipe the spinner with a kimwipe, specially the black band.
3. **Make sure the sample is seated properly in the sample gauge**, lift up and reseat if necessary. NMR tube bottom should touch the platform.
4. If the sample errors out, you can resubmit the sample (right click on the red spot and hit submit again).
5. Do check to see if the yellow light comes on when the sample is inserted into the magnet.
6. Do remove your samples from the auto sampler and make spinners available for other users.

Don't:
1. **Do not** use the red stop button to stop an acquisition: Go to "Acquisition" in the drop down menu and choose "Abort Acquisition" if you have to stop your run.
2. Do not remove your samples by clicking eject, stop acquisition (see above) and ensure that the reference sample goes back into the slot. Please let a WCAC staff know if you are not able.
3. Avoid "edits" copy and paste, as it tends to hang up the software.

Shimming:

One of the most important factors to get a good quality NMR spectrum is shimming. NMR spectroscopy demands a homogeneous magnetic field over the whole of the sample volume located within the probe's detection coil. If the field is not homogeneous, the resulting spectra will suffer from distorted line shape, which in turn leads to poor resolution and sensitivity. For this reason, the field has to be optimized to every sample that is introduced in the magnet. The process of adjusting the field's homogeneity is called "shimming". Shimming is achieved with the help of coils (called shims) located at strategic places around the sample in the magnet by applying different electrical currents to these coils to compensate. You can also use automatic gradient shimming on all our instruments. This procedure shims Z1 to Z5 simultaneously and usually achieves satisfactory results.

Modifying Spectral Parameters:

Before starting the acquisition, you may want to adjust the most common spectral parameters. These are found in the Acquire, Defaults parameter panel.

Select a different Spectral Width from the list or type in the chemical shift range. The default values for some nuclei can be very far from optimum for your sample or may not even encompass the values you expect. Narrowing the range will increase the digital resolution.

Select a different Spectral Width from the list or type in the chemical shift range. The default values for some nuclei can be very far from optimum for your sample or may not even encompass the values you expect. Narrowing the range will increase the digital resolution.

Select a Number of scans (number of transients: parameter nt) or type any number you want in the command line. For example, low sensitivity nuclei or diluted samples may require a large unknown number of scans. In such cases, enter a large number like nt=100000 and stop the acquisition later when enough signal has been accumulated.

To get a more accurate integration you may need to increase the Relaxation Delay (parameter d1). A Pulse Angle of 45 or 60 degrees is usually adequate for protons, but in order to get quaternary carbons in a carbon-13 spectrum it may help to lower the pulse angle to 30 degrees.

Other common parameters may be changed from the Acquisition panel or typed directly. For example, the acquisition time (at) determines the digital resolution of the spectrum; if you need high resolution fluorine spectra to accurately measure coupling constants, you should reduce the default spectral width and increase the default acquisition time. Do not change parameters that you don't understand and never increase pulse or decoupling powers.

Before starting the acquisition, you can check the time required by the experiment by clicking Show Time.

To create a queue of experiments on the same sample is to click New Study in the Holding vertical panel. You can then select several experiments from the buttons on the top of the vertical panel; customize them and run them with a single click on the Submit button. The sample will be automatically locked and shimmed, and the spectra will be transformed, plotted and saved.

Processing:

When you save a spectrum, the file is stored in the spectrometer's computer, usually in your vnmrj folder in /home/uniqname/vnmrsys/data. Your files will be accessible from any workstation in the lab.

To load a spectrum stored on disk into the current workspace, select **Open**... from the **File** menu, or click the **Open**... icon in the user tool bar just below the main menu (hover the mouse cursor over the icons to display their function). When you use this utility on any workstation in the lab, you will see links to all spectrometers. Clicking a link allows you to browse the files and load an FID stored in that instrument. If Process data on drag-and-drop is enabled (**Edit > System** settings menu), then the data will be Fourier transformed and processed automatically for you and it will be almost ready to plot: you may still need to check the referencing and phase as described below. If automatic processing is not enabled, you can do automatic processing from the menu **Process > Full Process**.

Processing an NMR spectrum generally involves applying a weighing function to the FID; applying linear prediction if needed; doing a Fourier transformation; phase correction; adjusting

the chemical shift reference; selecting integral regions; baseline correction if needed; and possibly getting a list of peak positions. Most of these task are done during automatic processing. However, it is important to understand how to do them manually because the automatic algorithms may not be completely accurate for all spectra.

The parameters for processing can be adjusted from the **Process** panels. Many of these parameters can be found in more than one panel and also in some of the vertical panels on the left hand side of vnmrj. The repetition is meant for ease of use but sometimes can be confusing. *If you are not sure about a parameter or its value, don't change it.*

Weighting function

Select a weighting function and its parameter(s) on the Weighting panel to be applied before Fourier transformation. The parameter for zero-filling is also found here.

Linear prediction

Linear prediction is also applied before Fourier transformation. Its parameters are located in the More 1D or More 2D panels. There is more information on linear prediction in a separate writeup.

Fourier transformation

Fourier transformation of 1D FIDs can be performed by typing wft, or by clicking **Transform All** in the **Default** or **Weighting panels**. You can also click **Transform** in the action bar. For 2D data, the appropriate button is called **Full 2D Transform**.

Interactive Display manipulation

Once transformed, the spectrum will be shown in the graphics window where it can be manipulated with the mouse. To this effect, there is a tool bar with drop down menus in the user tool bar where you can access all mouse functions, and there is also a vertical tool bar, shown below, on the right hand side of vnmrj with equivalent buttons. You can display the function of the buttons by hovering the mouse cursor over them. If you don't see a vertical red line on the spectrum, click the top button to get it. This

Display FID

Display 1D spectrum

Display one or two cursors

Show full spectrum

Reset to full display

Zoom in

Zoom out

Zoom mode

Pan & strecht mode

Show integrals mode

Show / hide scale

Show / hide threshold

Phase mode

Redraw the display

Return to previous toolbar

Show full integral

Define integrals

Adjust integral lvl/tlt

Hide integrals

Delete integrals

Adjust integral lvl/tlt

line is the vnmrj cursor and you are now in what we may call "normal display mode". Clicking with the mouse left button (left clicking) and dragging on the spectrum will move the cursor horizontally; middle clicking and dragging changes the intensity of the spectrum at the cursor position; and right clicking and dragging displays and moves a second cursor that can be used for zooming into a spectral region. You can also get a second cursor by clicking on the "Display one or two cursors" button on the toolbar. The middle button has another, special function: if you click on a region very close to the left edge of the display, dragging up and down with the middle button will move the vertical position of the spectrum instead of affecting its intensity.

To zoom into a region, first enclose it with the two cursors and then click . Or you can click and drag the mouse across the display to zoom in. If you choose this last button, you will enter into "zoom mode" (the mouse cursor changes into a magnifying lens) and you will have to click any other button, like the top button, to get out to normal display mode. Once you zoom into a region, clicking the pan and stretch button will take you to another operating mode where the mouse buttons have reassigned functions, but unfortunately in this case the mouse cursor doesn't change shape to alert you. In this mode, dragging the left mouse button pans the spectrum left or right; the middle button changes the intensity of the spectrum; and the right button expands the spectrum but in a bizarre way that is difficult to control: after you click the spectrum with the right button, any movement in any direction above the point where you clicked stretches the spectrum, and to compress it you have to move the mouse to a position below the point you initially clicked, possibly stretching it even more as you move the mouse down. Click the top

button in the toolbar to exit this mode.

Phase correction

The Free Induction Decay (FID) data set is a function of a complex number, the intensity, versus time. During Fourier transformation, this function is converted to a function of intensity (but still a complex number) versus frequency. The spectrum displayed on the screen should correspond to the real or absorptive
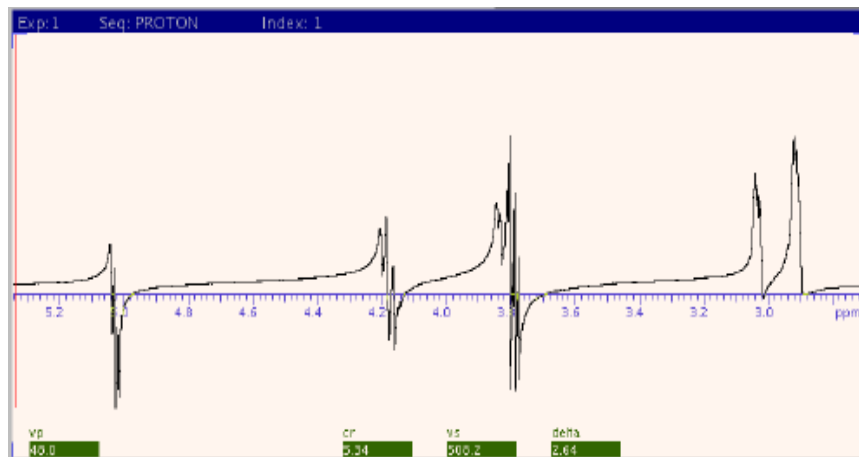
portion of the function, but it may contain "imaginary" or dispersive contributions as shown in the spectrum below. If you chose automatic processing or automatic phase correction (**Autophase** button in **Process, Default** panel), the spectrum will probably be phased correctly. But on occasions, you will need to fine adjust it manually.

To phase a spectrum, first click the Phase button to enter into phase correction mode. Left click on any portion of the spectrum close to its right edge and drag the mouse up or down until the rightmost peaks in the spectrum have only positive components; don't worry about the peaks on the left side of the spectrum yet. The right mouse button can also be used for fine adjustments. Then, left click close to the left edge of the spectrum and drag the mouse up or down until the peaks on the left side of the spectrum are completely in phase (positive intensity). Repeat the procedure if necessary until the full spectrum is properly phased. Click the top button on the toolbar to get out of phase correction mode.

Chemical shift referencing

Generally, the chemical shifts in the spectrum should be acceptable. By default, the chemical shifts are referenced using the solvent peak's deuterium signal. However, if you want to be more accurate, or if you have an unusual or non deuterated solvent, you may need to reference the chemical shifts manually using an internal standard like TMS. The reference can be set on the **Process, Default** or **Display** panels. Expand a small region around your internal standard, place the cursor in its center and enter the standard's chemical shift in the Reference cursor to field.

After acquisition, the chemical shift of any other nucleus will also be automatically referenced using the solvent's deuterium peak. But IUPAC recommends using the proton peak of the internal standards TMS for organic solvents and DSS for H_2O or D_2O. To reference any other nucleus in this manner, proceed as follows:

1. take a proton spectrum in workspace 1 and set the chemical shift of the internal standard (0 ppm for TMS or DSS)
2. take the spectrum of the nucleus of interest in another workspace, for example 2
3. type mref (1,2) in the command line to "move the reference" from workspace 1 to workspace 2.

Your spectrum is now referenced according to IUPAC without the need for cumbersome external references! (Pure Appl. Chem. 2001;73:1795-1818).

Integrating the spectrum

As a part of autoprocessing, integral regions are selected and the integrals' level and tilt are corrected. You may only need to normalize the areas by assigning a value to one of the integrals.

To do it, first click the button in the vertical toolbar to display the integrals; place the cursor over one of the integrals, and in the **Process, Integration** panel select **Normalize Area To: Single Peak**; enter a value in the **Integral Area** field and click the **Set Integral Value** button. The integrals' values can be displayed with the button **Display List of Integrals** or with **Show Integral Values**. If you want to select integral regions manually, first reset the regions previously defined, if any, with the **Clear Integrals** button. Then, select **Partial** under **Integral Display Mode**, click on **Interactive Resets** and define the regions by clicking on the spectrum over the left and right limits of each region that you

want to create. Always define the areas so they enclose some empty baseline on both sides. When you are done, click the top button on the vertical toolbar to get out of this "region selection" mode. It is also common to find that the whole integral "baseline" is not straight and horizontal: it may be tilted and may show a curvature. If that is the case, to get accurate integrations you will need to correct it manually. Select **Full** under **Integral Display Mode** and click the Integral

Lvl/Tlt button in the toolbar. Next, click on the *left side* of the spectrum and drag the mouse up and down until the integral baseline on that side of the spectrum is horizontal, then click on the *right side* of the spectrum and drag the mouse until the integral baseline is straight and horizontal across the spectrum. You may need to repeat the procedure to obtain optimum results. As usual, click the top toolbar button to get out of this operation mode. Notice that it is common to see some curvature or tilt close to one or both edges of the spectrum: this is usually unavoidable. Selecting **Partial, Full,** or **Off** under **Integral Display Mode** is equivalent to clicking the Show Integral Regions button , the Show Full Integral , or the Hide

Integrals button on the toolbar. Also notice that while the integrals are being displayed, the middle button will act on their intensity and not the spectrum's.

Baseline correction

There are two types of baseline correction available in vnmrj. The first one is a drift correction that only rectifies spectra with a spectral baseline that is straight but tilted
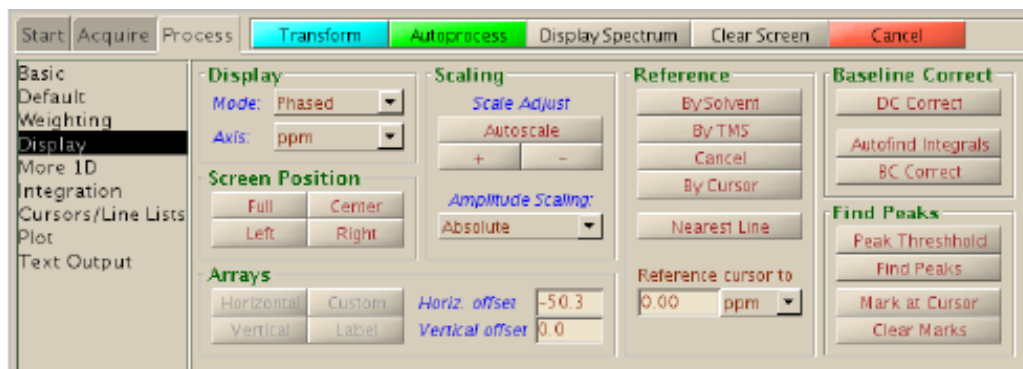
or that has non zero intensity.   The second one can correct a spectrum with a curved baseline. Both can be applied from the **Process, Display** panel. Click **DC Correct** to apply a drift correction, or **BC Correct** to apply a curvature correction. In the latter case, you need to have defined integral regions previously; click **Autofind Integrals** if you haven't defined regions before clicking **BC Correct**.

Getting a list of peak shifts

A list of peaks can be easily obtained with vnmrj. In the **Process, Cursors/Line** Lists click

Peak Threshold or the Threshold button on the toolbar ; move the horizontal line to the

lowest position in the spectrum where you want peaks to be listed, and click **Display Line**

List.