Processing NMR data in MacFID

Transporting file(s) to group iMac (see File Transfer cheat sheet in NMR lab)

1) Save file on Bruker NMR (360 or 500). 1H NMR files are best saved in their FID form. ^{13}C and ^{31}P NMR files will work better if they are fourier transformed on the Bruker and phased.
2) Make sure that the NMR Link program is running. Type CTRL X to change between memory regions. NMR Link runs in region 2. The cursor should show 2U. If it shows 2S, the program is not running. To restart the program, type **RUN NMRLINK** [RET]. If it shows 2S followed by S112, then the memory partition has crashed. Ask Dr. Belmore or other knowledgeable users how to reset the memory partition.
3) On PC in NMR lab, use the program **Get File** to transfer the desired programs from the Bruker NMR to that PC.
4) Use the **File Transfer** program to move the file from the PC to your bama account.
5) On the iMac, use **Fetch** to transfer the file from your bama account to the iMac. You must download the file using binary file transfer. Select Binary from the radio buttons on the bottom of the Fetch file transfer window. You will be asked what file name to give the downloaded file. Use a name that refers to the sample (i.e. khs-2-133a). If your spectrum is not a ^{1}H NMR spectrum, it is a good idea to add a letter indicating the nucleus (i.e. khs-2-133a-c for a ^{13}C NMR spectrum). You should store your files in a folder under your name in the NMR folder on the iMac hard drive. To make finding your files easier, keep a download folder that is for these files. That way they won't be mixed in with your processed data files. You should always keep a copy of both in case you need to go back and reprocess the data. For more information on using Fetch, see the online help menu in the program.

Importing into MacFID:

1) You must first import your Bruker file into the MacFID program.
2) Under the **File** menu, select **Import**.
3) In the window that opens, select Bruker as the instrument type
4) Click on the data file box. This will open a window to allow you to find your downloaded file. Once you find the correct file, click **open** in the window. This will return you to the original import window.
5) Click **OK**.
6) A window will open up with your FID or transformed spectrum (whichever you saved on the Bruker NMR).

Basic control of the spectrum;

1) To expand on a part of the spectrum, click once on the spectrum to give a cursor. Dragging this cursor will give a black area, which is the expanded area. Clicking on the black area will expand the spectrum.
2) To return to the whole spectrum, click on the small blue box containing your spectrum at the top right of the MacFID window.
3) The spectrum can be moved up and down relative to the x-axis using the scroll bar on the right side of the window.
4) In the main MacFID window, the two scroll bars on the left side control the amplitude of the spectrum. Adjust with the course and fine controls until your tallest peak of interest is at the top of the window. The fine control is opposite of the course control (i.e. the up arrow makes the peaks smaller).
5) To enter data into parameter boxes on the spectrum window (i.e. F1 in the main window), double click on the box. This will open a new window in which you can type the appropriate value.

6) It is usually best to set the display to high resolution. Under the **Display** menu, choose **Hi resolution display**.

Processing NMR data in MacFID (This assumes a ^{1}H NMR spectrum)

1) Fourier transform your data. Under **Commands**, click on **Bruker FFT**.
2) Phase your data. Under the **Options** menu, choose **Phase adjustment**.
3) You will see your spectrum with a cursor over the largest peak. Next to the window (left) are 2 scroll bars that control the phasing. The left control bar controls the area around the cursor. The right scroll bar controls the rest of the spectrum. Using the scroll bars phase the spectrum so each peak is has a flat baseline on each side of it. When satisfied with your phasing, click **OK**.
4) Baseline correct your spectrum. This may not always be necessary, but can fix a curvy baseline. From the **Options** menu, choose **Baseline Fix**. A number of **X**'s with dropdown arrows will appear on the spectrum. Move these so that they are all over flat parts of your baseline. Click **View** to see how the corrected spectrum will look. If you are satisfied with the View, then click **Apply** and **Exit** to return to main window.
5) To set your reference, expand on your reference peak. Place a cursor on the reference peak. In the **Display** menu, choose **Set Reference**. A window will open allowing you to enter the reference value. Select ppm as the units before entering the reference value.

Integration of your spectrum:

1) To integrate peaks, choose **Integral** from the **Options** menu. This will give you an integral icon in the upper left corner of your spectrum.
2) To place a new integral, click on the integral icon. This will give you a box, which can be dragged to your peak. It is usually best to expand on a small area of the spectrum and integrate it, then continue with different parts of the spectrum.
3) To expand the integral to the left or right, click and drag (left or right) on the small grey boxes at the bottom of the integral. It is usually a good idea to expand the integral over a wide range to ensure that it is level.
4) To fix the level, click and drag (up and down) on the open box in the upper right corner of the integral box. This will usually give an acceptably flat integral (over the baseline). If not, you can adjust the curve by holding down the **Option** key while dragging the open square box.
5) Repeat for every peak of interest.
6) Once you have placed integrals on each peak, choose one peak that you know the integral value of (If you do not know the structure of your compound, you can just set a small peak to 1.00). To set the value to the number of hydrogens, place cursor over the integral box of the desired peak and double click. In the window that opens up, set the assigned value to the number of hydrogens resulting in this peak. Click **OK** to accept this value.
7) To have all of the integrals drawn at the same scale, double click on your largest integral. You can make it larger or smaller by changing the multiplier value. Be sure the integral does not go off scale. Once you have a workable value (1 is usually fine), click the **set all** button and click **OK**.
8) I prefer to turn off the integral value labels, which can clutter up your spectrum. To do this, double click on the main integral icon. In the window that opens, click on the **Nil** button. Click on **OK**.

Set the spectral window:

1) It is good to print out your spectra, particularly ^{1}H NMR, using a standard window size. I use a window from 9.0 ppm to –0.5 ppm. By doing this you can overlay spectra taken at different times to compare peaks. Obviously, if you have peaks outside of this window you will have to adjust it. Also if your peaks only occupy a small region of this window, you may want to print out a second version that is expanded on the region of interest.

2) To adjust the print window accurately, double click on **F1** to change the left edge of the spectrum and **F2** to change the left side.
3) This works like an expansion, so if you need to go back to the whole spectrum, click on the blue spectrum box at the top left of the MacFID window.
4) When you print the spectrum (see below), MacFID will use whatever F1 and F2 values you have entered when you choose to print. Therefore, make sure you have the right boundaries before printing.

Peak picking:

1) To peak pick your spectrum, which you should always do, click on **Peak pick** under the **Options** menu.
2) The main window will change to show a black box along the bottom of your spectrum. The top of this box is the peak threshold. Any peak shorter than this will not be picked.
3) To adjust the threshold, double click on the **Level** box on the left of the window. Enter a value that will include all of the peaks of interest.
4) Click on **Apply**. If some peaks you would like to be picked are not, it is possible your level is too high. If this is not the case, then the noise factor is too high. This can be changed by double clicking on the **Noise** box and entering a lower value.
5) Repeat until all of the desired peaks are picked.
6) Once you've chosen the right **Level** and **Noise**, click on the label box to turn off the peak labels. These will clutter up your spectrum.
7) If you have fewer than 25-30 peaks, you can have them printed directly on the spectrum, by choosing print (see below) from the peak pick window. If you have too many peaks, you will have to print them on a separate page. Under the **file** menu, choose **Print**, which will open up a second menu. Choose **Peak pick**. Click print on the printer window that opens.

Printing the spectrum:

1) Before printing, enter a comment for your spectrum. Click on the notebook button in the upper left corner on the main window.
2) This will open a text box. Erase the text that is there (⌘ A, Delete). Type in the desired information. Required information for all of your spectra is the compound ID (i.e., khs-2-133a), the nucleus if not proton, the solvent, and the NMR strength. There is room for 4-5 lines of text. You may also want to add comments describing this sample. Click on the notebook button again to close.
3) To print your spectrum type ⌘ P (or choose **Print spectrum**, from the **Print** menu, which is in the **File** menu.
4) A print window will open up. Click on load and choose the **Default** file, to open the standard printing layout. This will include your spectrum, peak list (if peak pick is active), integral list (if integrals turned on), the comment, and the date. You may preview your spectrum by clicking on the **Preview** button. Make sure that all of your peaks are showing (if you want to print them on your spectrum) as well as all of your integrals. Also make sure that the integral or peak pick lists do not overlap with the spectrum. Clicking on the preview spectrum will close it returning you to the print menu.
5) If you are happy with the preview, click **OK**. In the printer window that opens click **Print**.

Saving your file

1) After printing your spectrum, close the window. You will be asked if you want to save your file.
2) Click **Yes** or hit return.

3) In the window that opens, find your NMR folder. You should not need to change the file name. It should be compound name with Import as the suffix (khs-2-133a.Import). Click **Save** to save file.
4) Periodically (i.e. monthly) back up your files to a zip disk.

The above describes the minimum steps necessary to get a good looking spectrum. There are a number of other tricks, many of which I don't know. If you get stuck, you can always read the manual, which is in the MacFID folder on this computer.