

I. Introduction to Spectroscopy Spectroscopy involves gaining information from the absorption, emission, or reflection of light from a sample. There are many other examples of spectroscopy in our experience, but three familiar real-life examples include:

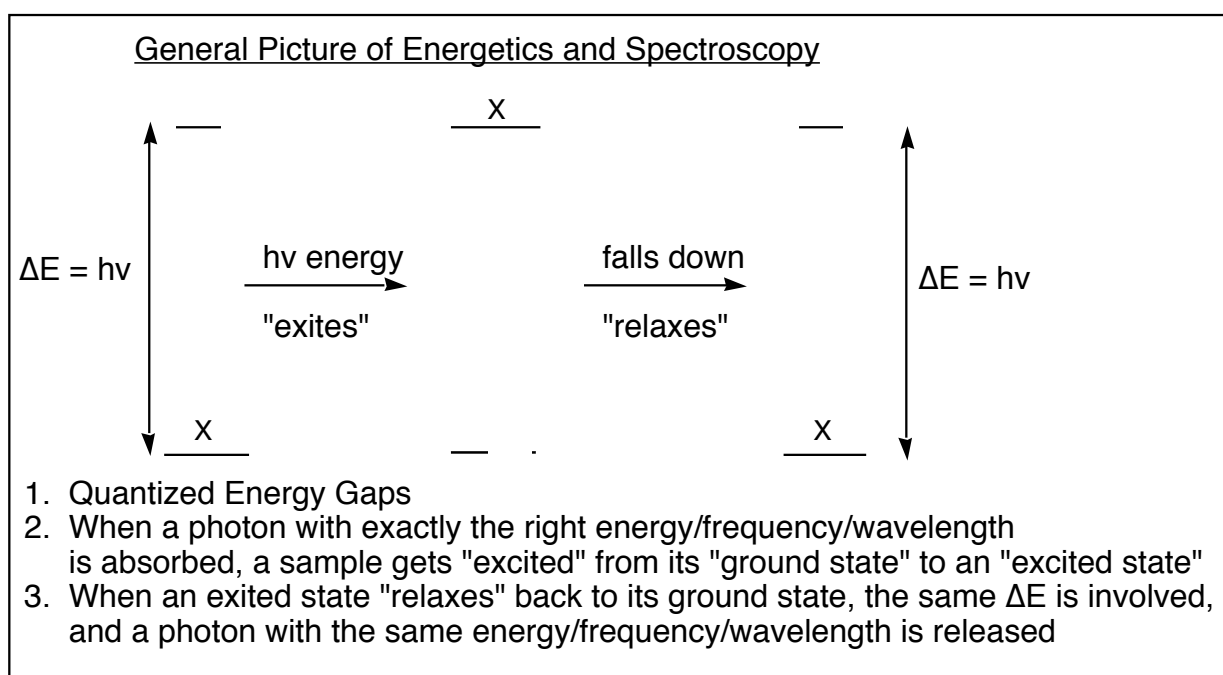
1. X-rays. Dense bone absorbs x-ray radiation.
2. Grocery store scanners. A monochromatic laser is either absorbed (black bar) or reflected (white bar). The simple black-or-white lines with their yes-or-no absorption-or-reflection response essentially produces a binary code, from which products and prices can be determined.
3. Stop lights. A lens is adjusted at timed intervals to enable emission of either green, red, or yellow light.

In organic chemistry, the most important type of spectroscopy is "NMR" ("Nuclear Magnetic Resonance" spectroscopy). NMR spectroscopy is routinely used for chemical analysis, whether that be to identify the structure of an unknown, to assess the purity of a product, or to determine ratios of isomers. This week we will use carbon-13 NMR; in two weeks we will use hydrogen NMR. Both of these will be used later in the year, especially during second semester lab. During second semester lecture, we will revisit NMR and spend time and a test on interpretation of NMRs. Magnetic Resonance Imagine ("MRI") is an important hospital application of NMR. (The name was changed from NMR to MRI because some patients were afraid of the word "nuclear" in NMR!)

II. General Aspects of Spectroscopy Physics The fundamental principles of chemical spectroscopy are illustrated below. Spectroscopy involves having quantized energy levels. You are familiar with the concept of quantized energy levels for electrons (1s, 2s, 2p, 3s, 3d etc.) and electron spins (spin up or spin down, but other things are also quantized (vibrational energies, rotational energies...)).

Given that there is an exact energy gap between two quantized energy states, a photon of precise energy must be absorbed in order to excite a molecule from the ground state. When an excited state relaxes back to the ground state, that same photon is released. By measuring the exact frequencies of photons that are either absorbed or emitted, we can measure ΔE . The quantity of photons can tell us about how much material is absorbing or emitting.

The chemist must then be able to interpret what the frequencies of the photons mean in terms of chemical structure.



III. NMR Physics

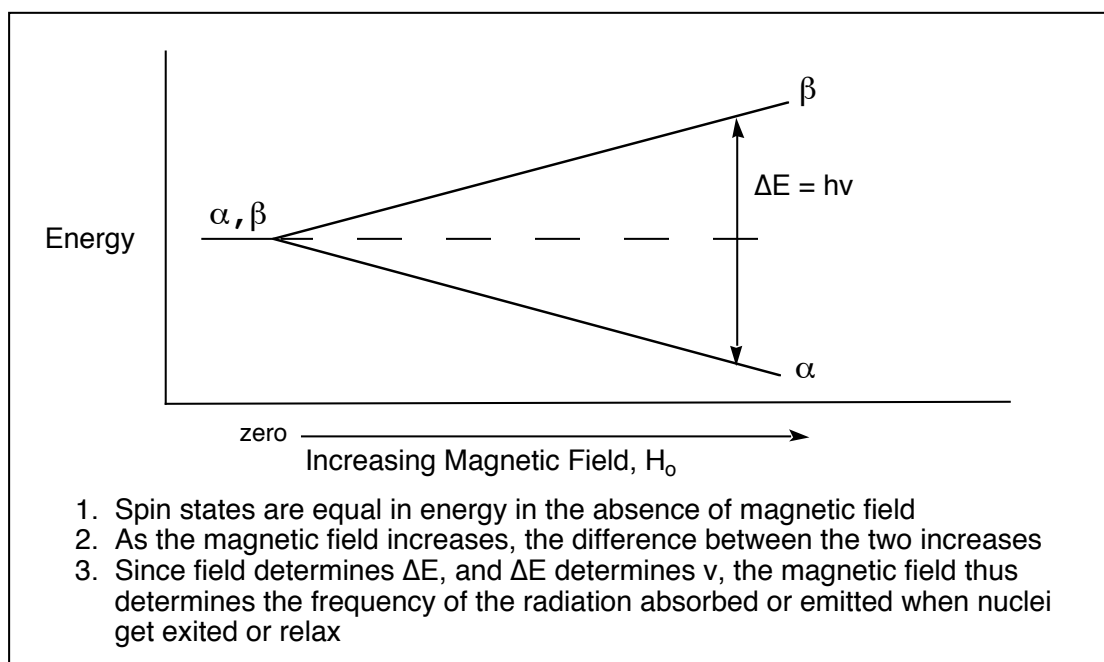
Certain nuclei (not all) have quantized “nuclear spins”. Being charged objects that spin, a result is that they magnetic. (A circular flow of charge or electricity always produces a magnetic field, according to the “right hand rule” of electromagnetism.) Nuclei that have quantized spin states are referred to as “NMR active”. Just as electrons have quantized spin states (spin up or spin down), NMR-active nuclei also have quantized spin states, spin up or spin down.

Some NMR-active nuclei: H-1, C-13, N-15, F-19, P-31, Si-29, Se-79, Sn-119

Some NMR-inactive nuclei: C-12, N-14, O-16

The list of NMR inactive nuclei is somewhat unfortunate for organic chemistry! We are largely interested in the chemistry of carbon and the 2nd row elements, but unfortunately the dominant isotopes for carbon, nitrogen, and oxygen are all NMR inactive! Fortunately at least carbon-13 is active. Although only 1% of carbons are C-13, that's still enough to give us useful information. Hydrogen is also NMR active, and can give us a lot of information (in two weeks...).

In the presence of an applied magnetic field, nuclear magnets can align with (spin down, α) or against (spin up, β) the field. The energy gap between these spin states is quantized, and depends on the strength of the magnetic field. (As with a bar magnet, the stronger the field, the greater the preference for the magnet to line up correctly...). To “excite” a nucleus from the more stable α state to the less stable β state, radiation with the correct photon frequency is required. When an excited nucleus relaxes back to the α state, a photon with that same frequency is emitted. Since magnetic field strength determines ΔE , and ΔE determines ν , the magnetic field thus determines the frequency of the radiation absorbed or emitted.



When an external magnetic field is applied, will all nuclei have the same ΔE and the same photon frequency? No!

1. Different nuclei (H-1 versus C-13) have very different ΔE . Thus an MRI can easily identify whether a particular nuclei is or is not present.
2. In different chemical environments, the same nucleus will have different ΔE .

The second point is the key to ¹³C NMR. Although the external magnetic field (applied by the spectrometer) may be the same, different carbons in a molecule experience or “feel” difference magnetic fields. This is due to the magnetic fields produced by local electrons and by other nuclei (because moving electrons function as “electron magnets” and moving nuclei function as “nuclear magnets”). The magnetic influence of local electrons and nuclei can reinforce or partially counteract the external field, so that every different carbon “feels” a different H_{actual} .

$$H_{\text{actual}} = H_{\text{applied}} + H_{\text{electrons}} + H_{\text{nuclei}} \quad H_{\text{actual}} \propto \Delta E \propto \nu$$

IV. The Actual Experiment

The actual steps in the experiment include:

1. Prepare the sample. For C-13, put in 5 drops of sample before diluting. For H-1, put in 2 drops of sample.
2. Insert the sample into the magnetic field.
3. “Lock” the magnet: (So that the magnetic field doesn't drift during the experiment)
4. “Shim” or “Tune” the magnetic field: Try to make it consistent from top to bottom, front to back, left to right. (This is very important for getting sharp lines).
5. Blast the sample with radiation to excite the nuclei. Rather than dialing through the different frequencies, a broad range of frequencies is applied so that all the carbon nuclei can get excited at the same time. After briefly blasting, the radiation is turned off.
6. Listen to the signals (actually in the radio frequency!) as the excited nuclei relax and release photons. (Many different signals with different frequencies are released simultaneously, each with it's own wave...)
7. Repeat the irradiate-then-listen sequence a number of times to build up the weak signal.
8. “Fourier Transform” (mathematical operation) to deconvolute the complex signal pattern resulting from the many overlapping frequencies. The Fourier Transform enables the computer to identify all the individual photon frequencies that summed up to give the total signal. An imperfect analogy would be to have every possible radio station broadcasting at the same time; then the Fourier Transform would essentially be able to identify and pick out each station one at a time and make sense of it.

Note: Many of these operations are best done by a computer. (The Fourier Transform especially!) Each of these steps also involves a number of software commands. So that you can acquire data and focus on chemical interpretation of the data, rather than being totally distracted by learning a lot of software commands, I have largely programmed the computer so that it can do most of this by itself. When it needs some input from the user, it will normally prompt you for input.

V. Interpreting C-13 NMR

While the physics of what happens is interesting, for the most part you the chemist will be engaged in interpreting the data that comes out at the end. This is true for the use of many instruments in science and health care. You need to learn some basic operational skills so that you can use the instrument safely and accurately. But being able to interpret the data is really what you need to be able to do at the end.

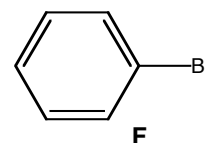
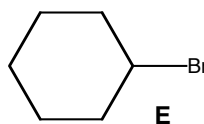
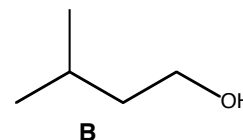
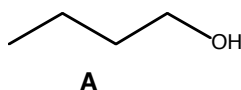
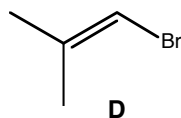
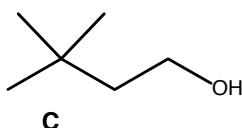
Summary of C-13 NMR Interpretation:

1. **Count how many lines** you have. **This will tell you how many types of carbons** you have. (Symmetry equivalent carbons can at times cause the number of lines to be less than the number of carbons in your structure.)
2. **Check diagnostic frequency windows** (“chemical shift windows”) of the lines **to provide yes-or-no answers regarding the presence or absence of key functional groups** in your molecule.

1. **Number of Lines and Number of Symmetry-Unique Carbons**

- a. Each “unique” carbon gives a separate line.
 - This is due to having different electronic environments, and because spinning electrons create magnetic fields that counteract or reinforce the applied field.
- b. Symmetry duplicates give the same line.
 - If due to molecular symmetry two carbons have exactly the same chemical environment, naturally they will absorb and emit exactly the same photon frequency, and give exactly the same line in the spectrum.

How Many Lines per Structure?
(Mark any symmetry duplicates)



2. **“Chemical Shifts” of the Lines** (This reflects the energies or photon frequencies/wavelengths associated with the lines.)

220-160 C=O carbonyl carbons, sp^2 hybridized
 160-100 C alkene or aromatic carbons, sp^2 hybridized
 100-50 C-O oxygen-bearing carbons, single bonds only, sp^3 hybridized
 50-0 C alkyl carbons, no oxygens attached, sp^3 hybridized

- Notice that sp^2 hybridized carbons come above 100, sp^3 hybridized come below
 - Notice that oxygenated carbons come higher than non-oxygenated analogs. An sp^3 -hybridized carbon with an oxygen comes higher than without, just as an sp^2 -hybridized carbon comes higher with oxygen than without
 - How do I process and use what I see from my Chemical Shifts?**
 - Check each of the four zones. Each one gives you a yes or no answer about the presence of absence of the featured group.**
 - Check 220-160. Do I have any carbonyl carbons or not? Easy yes or no question.
 - Check 160-100. Do I have any alkene/aromatic carbons? Yes or no! If I do, then how many? If I have two, I probably have an alkene! If I have four to six, I probably have a benzene!
 - Check 100-50. Do I have an oxygenated sp^3 carbon? Yes or no! Alcohols and esters will normally have one carbon in the 100-50 zone. Ethers will have two.
 - Check 50-0. I'll almost always have some lines there! But how many should tell me how many types of non-oxygenated sp^3 carbons I have.
3. **Signal Height/Size** When we get to 1H -NMR, signal size will be very important. For ^{13}C -NMR it isn't that crucial. There is considerable variance in height. But there are two patterns that can be somewhat helpful.
- Carbons without any attached H's are short. This is common for carbonyls (aldehydes are the only carbonyl carbons that have hydrogens attached) and for substituted carbons in a benzene ring.
 - Symmetry duplication multiplies signal height (if you have two copies of a carbon, the line will probably be taller than normal!)
4. **Subtracting the Solvent Lines: Don't Count the Triplet at 77** The sample is always diluted in a solvent. We will routinely use $CDCl_3$. When you use $CDCl_3$, it has carbons too! They give a 3-line “triplet” signal at 77, which is often kind of short (no H's attached.) Ignore this signal! Don't count it as three more unique carbons in your molecule! Don't conclude that you have three oxygenated sp^3 carbons!
5. **Subtracting the Reference Line: Don't Count the Line at 0** A reference chemical $[(CH_3)_4Si]$ is normally included that gets used to define where “zero” is. This zero marker is present all the time, but is not part of your actual molecule. Ignore this signal! Don't count the zero marker as an additional sp^3 carbon!
6. **How do I know what's a real line, from a carbon in my compound from an impurity that I should ignore?** No simple way! With experience you can often tell, but there is no automatic way to know. For today, if in doubt ask the instructor! The instructor will confirm which lines you should or shouldn't consider in doing your analysis

General: Work with a partner. Each pair should obtain spectra and identify at least two of the unknowns, whose identities will come from the list below. Each individual must have run one of the two samples. (If you don't have a partner, just run two yourself. All the better for you.)

Signing up for NMR Time: You can sign up on the web to reserve time in advance. Go to <http://www.mnstate.edu/jasperse/> NMR Signup is the link on the far left, and there is a login and password in the pink column. When you get into the schedule, click "Add an Event" to reserve a spot during the current week. If you want to reserve a spot in the following week, click the "Next week" arrow in the upper right corner, and then "Add an Event" there. A typical student should be able to finish within 15 minutes. If two of you are doing both samples together, you may prefer to sign up for 45 minutes combined, in case either of you has any trouble.

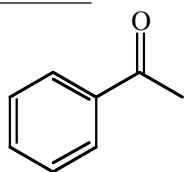
Preparing the sample

- Put in about 5 drops of sample, then add CDCl_3 solvent until it is about "three fingers" deep. (Typically about 1/3 full.) The volumes are not critical. Put a cap on the sample.
- Run the xmac "C13-notune".
- After you have analyzed your sample, pour the solution into the designated waste bottle, rinse your NMR tube with acetone, and return it to your drawer. Leave the tube open so that it can dry; put the cap on the closed end so that you don't lose it.

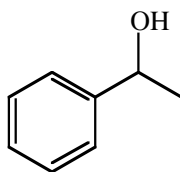
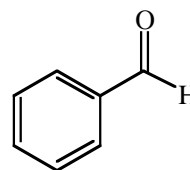
For each unknown, hand in the following:

1. For each of the 9 unknown candidates shown below, predict how many lines you'd get. Hand in this page.
2. Hand in a good, unexpanded NMR (label by hand)
-to get one for your partner, simply type "plot" twice!!
3. Hand in at least one horizontal expansion (whether needed or not) for your sample.
4. Give a structure identification. (write the structure directly on the NMR plot)
5. Explain the reasoning for your identification. This doesn't need to be very complicated. "It's the only candidate that had 6 unique carbons and had a carbonyl group" would be sufficient, or "Had one carbonyl, one C-O carbon, and four carbons total." Something that shows you can both count carbons and analyze the chemical shifts would suffice!

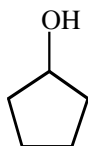
Unknown Candidates



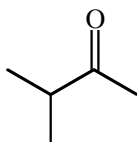
acetophenone

sec-phenethyl alcohol
(or DL- α -Methyl-benzyl alcohol)

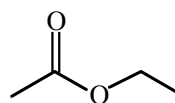
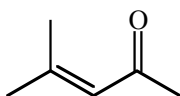
benzaldehyde



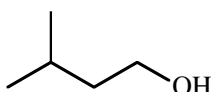
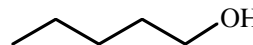
cyclopentanol



3-methyl-2-butanone

ethyl acetate
(ethyl ethanoate)

4-methyl-3-penten-2-one

isopentanol
(3-methyl-1-butanol)

1-pentanol

Running the Experiment, and What is the Instrument Actually Doing?

The overall experiment involves the following major steps:

1. Login to the Computer, Load the XWinNMR Program
2. Sample insertion
3. Calling up Standard Parameters. These include “acquisition parameters” (what experiment to run, how to run it at the instrumental level, how to tune the magnet if at all), how many “scans” to take, how to process the data once acquired, and how to plot the spectrum once the computer has it
4. Lock on Deuterium. A deuterium reference nucleus is required in the nucleus. The instrument determines the actual frequency of your photons by comparing them to the deuterium reference.
5. Tune the magnet. Inconsistencies in the magnetic field lead to line broadening. If not all nuclei experience the same magnetic field, their ΔE 's will vary unnecessarily, and thus their photon frequencies, and thus the lines will be wider. A well-tuned magnet is crucial to getting sharp spectra. The magnetic field tends to “drift” over time, and even sample to sample, so frequent tuning is necessary. Unfortunately the tuning often takes several minutes.
6. Acquire the raw data. The energy gap in NMR is in the radio frequency. To find which frequencies are absorbing/emitting, the instrument does not simply dial through all of the possible frequencies, the way that we do with a radio dialing through all the stations. Instead, the instrument irradiates all frequencies simultaneously, so as to simultaneously “excite” all the carbons at once. The irradiation then stops, after which the “excited” carbons “relax” to their ground state, and release their photons with specific frequencies. A detector “listens” to these signals. The signal is very weak, however. In order to build up signal, multiple “scans” are taken, each one consisting of an excite (irradiate)-relax (listen with detector) cycle. In today’s experiment, 64 “scans” are taken and the raw signal is then averaged. This takes several minutes.
7. Process the raw data. Since each radio signal is a wave, the total signal “heard” by the detector consists of multiple radio waves superimposed on each other. Just as a human ear can recognize one “sound” amongst a multitude of sounds, so can the computer. By conducting a “Fourier Transform”, the computer is able to deconvolute the complex raw signal and identify the specific frequencies involved. The spectrum is also phased so that the baseline looks level.
8. Plot

"lock" Locks the magnetic field on the deuterium signal of the CDCl_3 . To watch this process, highlight "windows" on the top, and then click "lockdisplay" signal under the heading

"tune" The electronics that define the magnetic field are adjusted to make it homogeneous. Inhomogeneity in the magnetic field leads to line broadening and line shortening, which lead to diminished resolution and signal/noise.

"copy all" Copies all of the settings needed for the experiment to be executed, for the data to be processed, and for the spectrum to be plotted.

"rga" "Receiver Gain Automation". Optimized a signal amplifier.

"zg" "Zero go". The sample is actually scanned and the raw data is acquired.

"efp" "ExponentialMultiplier-FourierTransform-Phase" The incomprehensible raw data is submitted to a complex mathematical treatment resulting in a meaningful, chemically sensible NMR spectrum!

"apk" "AutomaticPhase Korrection" (Bruker is a German company!) The spectrum is phased in order to give a flat baseline.

"abs" "Automatic Baseline Set" Sets a mark that enables automatic integration of proton spectra.

User Guide to ^1H , ^{13}C NMR

- Note: the default mouse button is the left button. Always use the left one unless told otherwise.
- For help, see Dr. Jasperse, Hagen 411-I.


1. Login

- a. double click on **jasperse** icon (or type in "jasperse")
(Research Users: You should use your boss's login.)
- b. Password "**chem355**" (need underline in between)
- c. double click on "**xwinnmr**"

2. Sample Insertion/Lock/Tune

- a. remove cap from spectrometer if needed, and then click **LIFT ON/OFF** key on upper lefthand corner of SCM keyboard (to right of computer) to lift lock sample
- b. place your sample in sample holder, adjust position using depth gage, and place in spectrometer [DO NOT PUT SAMPLE IN WITHOUT THE SAMPLE HOLDER! YOU WILL BREAK YOUR SAMPLE AND WRECK THE INSTRUMENT!]
- c. click **LIFT ON/OFF** key on SCM keyboard to lower sample

3. Acquiring the spectrum

- a. type "**xmac**"
 - a listing of suggestions will come up
 - at present, all of these assume CDCl_3 as solvent
 - instructors/researchers, these can be easily customized for you needs. See Jasperse
- b. select the experiment of interest, normally **ah1-tune** or **c13-tune** or **c13-notune**
 - Note: if you are going to run both ^1H and ^{13}C on the same sample, you don't need to tune twice. Run "ah1-tune" first for hydrogen, then "c13-notune"
- c. when asked for file name information, type your name into the name box and
- d. type "**chem355**" into the "user" box.
- e. click **SAVE**
- f. click **COPY ALL** when the box comes up
 - The computer will now do everything for you: read in the correct parameters, lock, tune specified, adjust the receiver gain, acquire the spectrum, phase the spectrum, and store the phase information for automatic integration.
- g. When "ns = 8 (or 128)" box pops up, hit return to accept default, or else enter something different for the number of scans
- h. wait patiently until either an "**xmac:finished**" or "**abs finished**" message appears
 - Hopefully this whole process will take less than 5 minutes for proton or less than 8 minutes for carbon spectrum. If 8 minutes have passed and still incomplete, see Jasperse...
- i. click on the  icon (upper right corner of the icon group) to adjust the vertical scale of the viewed spectrum. (For example, if your baseline looks flat, this will fix it!)


4. Plot

- a. click **PLOT** icon
- b. hit return in response to any boxes that appear
- c. To do **horizontal expansions**, **manual integrations** or **vertical expansions**, see instructions on page 2.

5. Exiting:

- a. Replace your sample with the default sample, as described in part 3.
- b. Type "lock cdcl3"
- c. type "**exit**"
- d. Say OK if it asks you anything about closing things
- e. put cursor outside of any boxes into the blue area, then press the *right* mouse button, click **Logout** and click **Yes**.

1. Plotting **Horizontal Expansions**


- Make sure that the cursor is somewhere on the spectrum.
- Click the **left mouse button**. You will now get a doubled arrow.
- Move the doubled arrow to the left end of the area you want to expand and click the **center mouse button** to define the **left boundary**.
- Move the doubled arrow to the right end of the area you want to expand and again click the **center mouse button** to define the **right boundary**.
- click **PLOT** icon
- To get back to the full expansion, click the  icon
- To get out of the "doubled arrow" mode, click the **left mouse button**

2. **Manual Integration**

- click **INTEGRATE**
- define the regions of interest (see horizontal expansion instructions above)
- click **RETURN** and save your integral regions
 - Sometimes you may wish to improve the "flatness" of the integral, or you may wish to assign calibration values of your own choosing. Do the following:
- put the arrow within the region of your integral, and click the **left mouse button**. The integral under consideration will then get a star by it.
- click **CALIBRATE** and respond accordingly
- adjust the **BIAS** in order to get the left side of the integral level
- then adjust the **SLOPE** to get the right side of the integral level

3. Reducing the noise in noisy, dilute ^{13}C spectra. "**Power Spectrum**" .

Do not use for ^1H spectra!

- After getting the normal spectrum, type "**ps**"
- click on the  icon (upper right corner of the icon group) to adjust the vertical scale of the viewed spectrum.
- click **PLOT** icon
- Note: The "ps" command can make plots look prettier, by de-emphasizing noise. It does so by squaring all signals, however, so it will also de-emphasize small peaks that are real. In addition, by changing the relative sizes of peaks, it is incompatible with integration.

4. **Vertical Expansions**

- Type "**cy**" and increase or decrease the default value as you see fit. Doubling will double the printed heights, tripling will triple the printed heights, etc.
- At default, cy=14, and is set so that the tallest peak in the spectrum will be 14cm tall. Thus, if you are wanting to expand a peak that is too tall, you need to multiply the cy as needed.

5. Manual Phasing

- click **PHASE**
- Click **BIGGEST**
- Click **PHO**, and keep finger held down
- drag, to adjust phase of biggest, marked peak
- Click **PH1**, and keep finger held down
- drag, to adjust phase of peaks distant from biggest
- click **RETURN**
- type "**abs**" if you want integrations to be automatically printed as a result

6. Printing Titles

- type "**setti**" (for "set title")
- delete existing title and type in new one
- click **save**
- click **quit**
- type "**title**"
- choose **yes**