Varian NMR Instructions - 2D

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General Information

The University of Minnesota Chemistry Department NMR Laboratory is located in 194 and 196 Kolthoff Hall and is operated by Dr. Letitia Yao (Tel: 625-8374; e-mail: yaoxx006@umn.edu) and Dr. Steve Philson (Tel: 626-0297; e-mail: philson@nmr.chem.umn.edu). The laboratory contains 6 NMR spectrometers:

Varian INOVA-500	(VI-500)
Varian INOVA-300	(VI-300)
Varian INOVA-300	(VXR-300)
Varian UNITY-300 with automatic sample changer	(VAC-300)
Varian UNITY-200 with automatic sample changer	(VAC-200)
Varian UNITY-Plus 400	(VMX-400)

This document describes operation of the three hands-on Varian NMR spectrometers (VI-500, VI-300, VXR-300, and VMX-400) for 2D experiments. It is assumed that users will already have a good working knowledge of the instruments before running these experiments.
- The standard probe for the VI-500 is a triple-resonance (HXP) indirect detection PFG probe that can do ^1H, ^{13}C, and ^{31}P acquisition and is equipped with pulsed field gradients. (The X channel is tuned to carbon.)
- The standard probe for the VXR-300 is a 4-nucleus probe that can do ^1H, ^{13}C, ^{19}F, and ^{31}P and also has gradients.
- The standard probe for the VI-300 is a broadband probe that can do ^1H and ^{13}C. There are no gradients.
- All three spectrometers are equipped with additional probes that can be tuned to most other nuclei. Contact Letitia for information on scheduling time to do other nuclei.
- Three spectrometers are equipped for temperature control. *Separate training is required for use of the variable temperature equipment.*
    The VT range of the standard PFG probe in the VI-500 is -20°C to +60°C.
    The VT range of the standard probes in the VI-300 and VMX-400 is -100°C to +100°C.
- Several Sun workstations are located in the NMR lab for off-line processing of data. Use these workstations for processing and plotting of data - our goal is to maximize the amount of time the spectrometers are busy collecting data.

Two-Dimensional Experiments: Descriptions and Applications

Two-dimensional NMR experiments can provide valuable information not easily obtained from 1D experiments. 2D experiments provide increased resolution and additional information by spreading peaks out in a second frequency dimension and by correlating sites in a molecule that interact via J-coupling, sites that are less than 5 Å apart through space (NOE), or sites that are connected by chemical or conformational exchange.

References:
Braun, S.; Kalinowski, H.-O.; Berger, S. "150 and More Basic NMR Experiments"
Claridge, T.D.W. "High-Resolution NMR Techniques in Organic Chemistry"

I. Homonuclear Correlation Experiments

The most common 2D experiments are correlation experiments that provide information about nuclei which interact through some mechanism like J-coupling, NOE, or exchange. For example, a proton COSY experiment correlates protons that are directly J-coupled; a proton NOESY experiment correlates protons which interact through space via the nuclear Overhauser effect.

I. A. Through-Bond

I. A. 1. COSY: Correlation Spectroscopy

The simplest and often most useful 2D experiment is the absolute value COSY experiment. Crosspeaks in a COSY spectrum arise from pairs of protons that share a mutual scalar coupling. Correlations usually occur between geminal or vicinal protons (2- or 3-bonds) with coupling constants in the range of 3 to 15Hz. Long-range couplings (allylic or W-coupling) may also be seen in some cases.

I. A. 2. DQF-COSY: Double-Quantum-Filtered COSY

The Double-Quantum-Filtered COSY (DQF-COSY) provides information that is virtually identical to that provided by the absolute value COSY. The DQF-COSY, however, is generally run in phase-sensitive mode, generating narrower peaks than in the absolute value COSY. This makes crosspeaks near the diagonal easier to distinguish. The DQF-COSY also has the advantage that singlet peaks (including solvent peaks) are eliminated or reduced in intensity. The price paid for these advantages is a loss of sensitivity by a factor of two over the AV-COSY. This means that for comparable signal-to-noise, the DQF-COSY must be run four times longer than the AV-COSY. In many cases where the sample is concentrated, this is not an issue, and the DQF-COSY provides better results in the same amount of time. The other disadvantage of the DQF-COSY arises from the increased complexity of processing phase-sensitive data.

I. A. 3. TOCSY: Total Correlation Spectroscopy

TOCSY spectra are similar to COSY spectra in that they contain information about J-coupled protons on a molecule. TOCSY spectra, however, do not simply display crosspeaks between pairs of J-coupled (geminal or vicinal) protons, they also display crosspeaks between pairs of protons that are not directly J-coupled but are in the same J-coupling network or spin system by relaying coherence from one proton to the next within the chain. A short mixing time may show only COSY-type peaks (2-3 bonds), while longer mixing times will result in multistep transfer (4+ bonds). Such information is particularly useful if a molecule is composed of several subunits in which the protons within each subunit form an isolated spin system. One of the most common uses of the TOCSY experiment is in protein structure determination as it enables the identification of individual amino acids in the protein.

I. B. Through-Space

I. B. 1. NOESY: Nuclear Overhauser Effect Spectroscopy

NOESY spectra contain crosspeaks that reveal NOEs between protons in a molecule. NOEs are caused by dipolar cross-relaxation between nuclei in a close spatial relationship. Due to the r^{-6} dependence of NOEs on interproton distance, crosspeaks appear only if protons are less than 5 Å apart. The NOE interaction does not depend on intervening bonds like J-coupling does, so NOEs can be seen between protons on different parts of a molecule. This makes NOEs extremely useful in determining the conformation of a molecule.

One limitation of NOESY spectra is that the intensities of the crosspeaks depend on the relationship between molecular rotational correlation time (τ_c) and the spectrometer proton frequency (ω_0). Therefore, crosspeaks in NOESY spectra may change in intensity at different magnetic field strengths and in different solvents. Even more importantly, it is sometimes not possible to observe crosspeaks in a NOESY spectrum even when the protons are less than 5 Å apart. This usually occurs for molecules with molecular weights of a couple thousand where $\omega_0\tau_c \sim 1$. For small molecules where $\omega_0\tau_c \ll 1$, crosspeaks are observable in a NOESY spectrum that are opposite in sign to the diagonal peaks. For large molecules of several thousand molecular weight where $\omega_0\tau_c \gg 1$, crosspeaks observed in a NOESY spectrum have the same sign as diagonal peaks. For compounds in the intermediate molecular weight category where NOESY crosspeaks aren't observable, it is necessary to run a ROESY experiment (described below) to observe NOEs (or ROEs).

Proper selection of the NOE mixing time is extremely important because NOEs require time to build up. The mixing time is dependent on the molecular rotational correlation time (τ_c) which is loosely associated with molecular weight. Mixing times comparable to $\sim 2/3*T_1$ (0.5 to 2s) provide maximum NOE enhancements, although such measurements may not be quantitative. Also, if the 90 degree pulse width is incorrect or the mixing time is too short, "COSY-type" (antiphase) crosspeaks may appear in the spectrum. True NOE peaks are pure absorptive.

I. B. 2. ROESY: Rotating Frame Nuclear Overhauser Effect Spectroscopy

ROESY spectra are essentially identical to NOESY spectra. The advantage of the ROESY experiment over the NOESY experiment is that NOEs can be seen in ROESY spectra regardless of molecular weight. The NOE crosspeaks in ROESY spectra are always opposite in sign to the diagonal peaks. The ROESY experiment is

more complex and more difficult to perform than the NOESY experiment, so you should usually try NOESY first. As NOESY spectra can have COSY-type artifacts, ROESY spectra can show TOCSY-like artifacts.

The ROESY pulse sequence can also be used to measure exchange. In ROESY spectra, exchange crosspeaks will always be the same sign as the diagonal peaks. This can be an advantage over NOESY if you are trying to measure exchange in large molecules, since the NOE peaks and the exchange peaks will have opposite sign.

II. Heteronuclear Correlation Experiments

2D experiments involving more than one type of nucleus are referred to as heteronuclear experiments. In general, one nucleus is proton, and the other is usually carbon or nitrogen. Direct detection experiments observe the heteronucleus directly. Indirect or inverse detection experiments observe the heteronucleus indirectly, via transfer of magnetization to proton. The sensitivity of indirect detection experiments is greatly enhanced due to the more abundant proton nucleus.

Three advantages of heteronuclear experiments are the ability to assign carbon spectra based on their attached proton chemical shifts, dispersion of the proton chemical shift into another dimension, and the identification of diastereotopic geminal protons.

II. A. Direct Detection

II. A. 1. HETCOR: Heteronuclear Correlation

The HETCOR experiment is a ^{13}C-^1H correlation experiment which generates crosspeaks resulting from proton-carbon nuclei that are connected through a single bond. The directly detected nucleus in the HETCOR experiment is carbon, thus this experiment suffers from the same insensitivity as a one-dimensional carbon spectrum. The HETCOR has largely been replaced by the inverse detection experiment, HMQC. Advantages of the HETCOR experiment are that it is less prone to artifacts than the corresponding HMQC and provides high resolution in the ^{13}C dimension, however a high price is paid in sensitivity (or lack thereof).

II. B. Indirect Detection

II. B. 1. HMQC: Heteronuclear Multiple Quantum Correlation

The HMQC is an inverse detection experiment in which the directly detected nucleus is proton and the carbon nucleus is detected indirectly. While it gives the same information as a HETCOR experiment, the sensitivity is greatly enhanced due to detection of the more abundant nucleus. The essence of the HMQC experiment is the cancellation or elimination of the signals from protons attached to ^{12}C, leaving only signals from protons attached to ^{13}C, contributing to a ^1H - ^{13}C chemical shift correlation spectrum.

Unfortunately, the residual uncanceled signals from protons attached to ^{12}C show up as stripes parallel to the f_1 axis at the frequency of each ^1H peak; these artifacts are larger for peaks with long T_1 (solvent peaks or methyl groups). Other artifacts include axial peak artifacts at $f_1=0$ (the center of the spectrum in f_1) or at the edges of the spectrum in f_1, at f_2 frequencies corresponding to each ^1H peak and a "0,0" artifact in the exact center of the spectrum.

II. B. 2. HSQC: Heteronuclear Single Quantum Correlation

The HSQC is a Heteronuclear Overbodenhausen experiment using REVINEPT and gives essentially the same information as the HMQC experiment. The difference between the two experiments depends on how the coherence evolves during the evolution period, as either multiple quantum (HMQC) or single quantum (HSQC) coherence. Due to its improved resolution, the HSQC is sometimes superior in cases where the ^{13}C spectrum is crowded. It is often used instead of HMQC for ^{15}N-^1H correlation, although this requires isotope enrichment. The HSQC is more sensitive to experimental defects (such as inaccuracies in the 90 degree pulsewidth) than the HMQC.

II. B. 3. HMBC: Heteronuclear Multiple Bond Correlation

The HMBC experiment is a multiple bond HMQC. In other words, long range ^{1}H - ^{13}C couplings can been seen over two or three bonds. Suppression of the one bond coupling is not perfect, so the experiment is run without decoupling to distinguish the residual one-bond couplings. HMBC experiments can often provide information about carbons that have no attached protons (carbonyls or quaternary carbons) or connectivity information across nitrogen or oxygen atoms. Unfortunately, the lack of a correlation is not definitive, as heteronuclear coupling constants may approach zero.

III. J-Resolved Experiments

In a normal 1D spectrum, chemical shift and coupling information are observed along the same axis and may be obscured by overlapping multiplets. J-resolved experiments separate these two parameters along different axes. These experiments can be very useful for accurate measurement of proton-proton or proton-carbon coupling constants, including long-range proton-carbon coupling constants. There are homonuclear and heteronuclear versions.

III. A. HOM2DJ
The homonuclear 2DJ experiment has mostly been replaced by the COSY type experiments. However, it is a very quick and easy method to obtain your J-couplings. Spectra correlate the chemical shift on one axis and the coupling constant on the other.

III. B. HET2DJ

This is the heteronuclear version of a J-coupling experiment. Chemical shift information is present along the F2 axis and the C-H coupling constant shows up on the F1 axis.

IV. Pulsed Field Gradients (PFG)

Gradients are available on the VI-500 and the VXR-300 spectrometers. In 2D applications, the use of gradients is advantageous in that it reduces the time required for acquisition. Pulsed field gradients dephase coherences along the z-axis. In gradient-selected experiments, judicious application of gradient pairs can select (and rephase) the desired coherences while suppressing others, eliminating the need for phase-cycling and thus shortening experiment times. The majority of experiments utilizing gradients are simply modifications of existing methods that employ phase-cycling for coherence selection. In simplistic terms, phase-cycling involves the repetition of a pulse sequence using different phases for the pulses. The data is then combined such that the desired signals add constructively, while the undesired ones cancel out. For the required selection to occur, the complete phase cycle must be completed, thus the requirement in non-gradient experiments for minimums (and multiples) of 4 or 8 scans. For example, a standard COSY requires a minimum of 4 scans, taking 25 minutes, while the corresponding gradient-COSY can be performed with a single scan in 5 minutes as long as the signal intensity is sufficient in one scan.

Gradients can also be used for shimming and suppression of solvent peaks. This is discussed elsewhere.

Two-Dimensional Experiment Setup

1. Insert sample, lock and shim

2. Turn Spinner Off

    Turn the spinner off (in the [Acqi] window). Adjust the non-spinning (x,y) shims if the lock level drops dramatically (>10%) when the spinner is off.

3. Take a 1D Spectrum of the Directly-Detected Nucleus

    Take a 1D spectrum with one scan (**nt=1**) of the nucleus you will be directly detecting (usually H1).

4. Set Spectral Width

    a. Place the cursors approximately 0.5-1.0 ppm on either side of the left- and rightmost peaks in the 1D spectrum, and type **movesw**.

    b. Take another 1D spectrum with this reduced spectral width, phase, and reference it.

    c. Save your spectrum for printing purposes: **svf('filename')**

5. Set Up Presaturation (optional) to remove solvent peak

    a. Type **gain='y'** to set **gain** to the value found by autogain (**gain='n'**).

    b. Place cursor on peak to be presaturated and type **sd**. This sets **dof**.

    c. Set **dm='nyn' dpwr=20 homo='y' d1=0 d2=2**.

    d. If necessary adjust **dpwr** and/or **dof**. Do not use **dpwr** > 40.

6. Calibrate 90° Pulse

    For experiments with multiple pulses, the value of the 90 degree pulse is critical for success of the experiment. The 90° pulse width is the duration of radio-frequency pulse at a given transmitter power (**tpwr**) that gives maximum signal from a single pulse. In practice, the 360° pulse width is the easiest to find as it is not as prone to artifacts. *The 360° pulse width will be the null point going from negative to positive intensity.*

    a. Determine the expected 360° pulsewidth (4 times the 90° pulse). If you have no idea where the 360° pulse should be, you can run an array of 20 steps, starting at 3, and incrementing by 4, as follows:

    b. Type **array**.

        The spectrometer will prompt you with the following questions:
   parameter to array: **pw**
                enter number of steps in array: **20**
                enter starting value: **3**
                enter array increment: **4**

        Upon completion of this script, a display of your arrayed values will appear on the screen. If not, go to the [**Text**] window and type **da**.

    c. Set **nt=1** and type **ai** to enter the absolute intensity mode.

    d. Run an experiment (**ga**).

    e. Type **dssh** to display all the spectra and **dssl** to display the spectrum number. (Optional: adjust the vertical scale (**vs**) and vertical position (**vp**) so all the peaks are on scale, eg., **vs=vs/2** and **vp=50**)

The 360° pulse will be the null going from negative intensity to positive intensity.

Shown below are the results from an array of **pw** values displayed with the **dssh** command. The 360 degree pulse is somewhere between the 7th and 8th spectra.
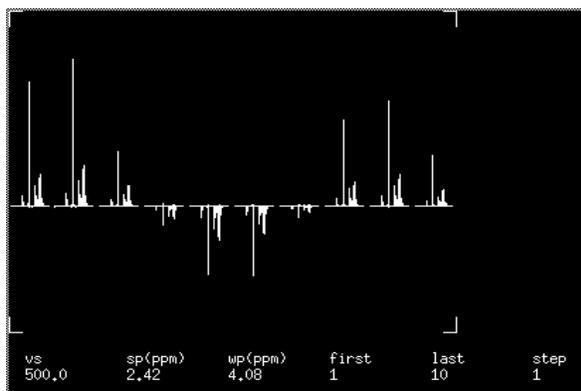
f. Adjust your array to find a more accurate value of **pw** once you have a general idea of where it is. In the above example, you would want to array again between the 7th and 8th spectra, using smaller increments. See step b.

g. Set the 90° pulse to this value: **pw=*360°*/4** where 360° is the value you found in step f. **pw?** will tell you the value. For example, if you obtain a 360° pulse width of 28.4 microseconds, the 90° pulse width will be 7.1 microseconds. Let the computer do the math by typing **pw=28.4/4**.

h. Run another 1D experiment with this value of the 90° pulse.

i. To make the spectrum full scale again, type **f full**. Also reset **vp=12** if you changed it in step e.

7. (TOCSY and ROESY only) Calibrate spinlock pulse

If you are running a TOCSY or a ROESY experiment, you will need to calibrate another 90 degree pulse at a lower power level.

a. Reduce the power level: **tpwr=48** for organic solvents, **tpwr=52** for D_2O.

b. Calibrate the 90° pulse according to step 6. The expected 360° pulse will be around 100 μs.

8. Take a 1D spectrum of the Indirectly-Detected Nucleus (Heteronuclear Experiments Only)

a. Join another experiment (e.g., **jexp2**)

b. Take a quick (**nt=1**) 1D spectrum of the indirectly-detected nucleus (usually C13).

c. **movesw** to reduce the spectral width in this dimension.

d. Take another quick 1D spectrum. Reference this spectrum. Save it if desired.

9. Setup of the 2D Experiment

a. Join the experiment where the 2D experiment will be run, e.g., **jexp3**
or **[Main Menu]** - **[Workspace]** - **[exp3]**.

b. Move the parameters from the experiment where you did the setup of the *directly*-detected nucleus (step 4) into this experiment. For example, **mp(1,3)** will move the 1D parameters from experiment 1 to experiment 3.

10. Type the Macro for the Desired 2D Experiment and Change Experiment-Specific Parameters

HOMONUCLEAR EXPERIMENTS
- **gcosy**—correlated spectroscopy (gradient version) / **cosy** for non-gradient version (VI-300)
    nt can be one scan for a gcosy; must be a multiple of 4 scans for a non-gradient cosy.
    Change p1 to a 45º pulse if you want to decrease the size of the diagonal and emphasize active coupling partners.
    The following parameters pertain only to the non-gradient version:
      relay=0 for a normal absolute value COSY (3-bond coupling)
      relay=1 for a relay COSY (4 bonds)
      relay=2 for a double-relay COSY (5 bonds)
              As relay increases, the sensitivity decreases.
      tau = propagation time; usually 0, but you can set **tau=0.2** to emphasize long-range couplings.
    If presaturation is desired, set **dm='ynn'** and verify that **dpwr** and **dof** are set to the values found in the 1D setup of presaturation (step5).

- **dqcosy**--double quantum filtered cosy / **gmqcosy** for gradient version
    If presaturation is desired, set **presat=1** and verify that **dpwr** and **dof** are set to the values found in the 1D setup of presaturation (step 5).
    nt = multiple of 8 if phase=0; multiple of 4 if phase=1,2 or 3.
    (There are no peaks in the first fid.)
    For the gradient version (gmqcosy):
      gt1=0.003 for organic solvents (default)
      gt1=0.008 for aqueous solvents

- **tocsy**--total correlated spectroscopy
      mix - isotropic mixing time. Typical values are 30 to 80 milliseconds. As mixing time increases, the amount of magnetization transfer also increases.
      p1 - 90° pulse length at full power **p1lvl** (from step 6)
      p1lvl - ^{1}H pulse power for the first 90° pulse **p1**. Should be the same as for a 1D proton experiment (from step 7)
      pw - 90° pulse length at reduced power **tpwr** (from step 7 for spinlock pulse)
      tpwr - ^{1}H pulse power for the trim pulse and spin-lock pulses (from step 7)
    If presaturation is desired, set **presat=1** and verify that **dpwr** and **dof** are set to the values found in the 1D setup of presaturation (step 5).

- **noesy**--nuclear overhauser effect spectroscopy
      mix - NOE mixing time for magnetization exchange. Typical values are 0.2 seconds for macromolecules to 0.5 - 1.5 seconds for small molecules (default **mix=0.5**), or on the order of $2/3*T_1$ of the molecule. A mixing time that is too short will not allow the NOE to buildup; one that is too long results in spin diffusion. If the mixing time is too short, "COSY-type" (antiphase) crosspeaks may appear in the spectrum. True NOE peaks are pure absorptive.
      phase=1,2 is suggested. Use **phase=0** for P-type peak selection for av display.
      nt = multiple of 8 for phase=1,2 or 3. Multiple of 16 for phase=0
    If presaturation is desired, set **presat=1** and **dm='nyyn'** and verify that **dpwr** and **dof** are set to the values found in the 1D setup of presaturation (step 5).

- **roesy**--rotating frame nuclear overhauser effect spectroscopy
      mix - NOE mixing time. For ROESY, this is the length of the spin-lock. Typical values are 0.2 seconds for macromolecules to 0.7 seconds for small molecules (default **mix=0.4**).
      ratio - spin-lock field strength. As **ratio** increases, effective spin-lock decreases. If too short, TOCSY peaks begin to appear. If too long, the protons are not locked in the rotating frame and therefore do not exchange magnetization via cross relaxation in the rotating frame. **Ratio** should be increased until the protons of interest begin showing sufficient amplitude and phase stability. Try values of 10-12.

p1 - 90° pulse length at full power **p1lvl** (from step 6)

p1lvl - ^{1}H pulse power for the first 90° pulse **p1**. Should be the same as for a 1D proton experiment, i.e., **p1lvl=58** (from step 6)

pw - 90° pulse length at reduced power **tpwr** (from step 7 for spinlock pulse); use 30º pulse (or 1/3 of the value from step 7)..

tpwr - ^{1}H pulse power for the trim pulse and spin-lock pulses (from step 7, e.g., tpwr=48)

phase=1,2 is recommended.

rocomp='y'

sspul='y' for homospoil before d1, if recycle delay is less than T_1

nt = multiple of 4

If presaturation is desired, set **presat=1** and verify that **dpwr** and **dof** are set to the values found in the 1D setup of presaturation (step 5).

HETERONUCLEAR EXPERIMENTS

- **hetcor**--heteronuclear correlation experiment; carbon detected (VI-300 only)

  Do you really want to do a hetcor? Why don't you try the hmqc or ghmqc instead?

  Hetcor requires as much or more sample as a 1D carbon experiment.

    nt = multiple of 4

    j1xh – average one-bond X-H coupling constant

    For long-range hetcor

    jnxh – set to average 2 or 3-bond coupling constant

    hmult='y'

- **ghmqc**--heteronuclear multiple quantum correlation; proton detected (gradient version) / **hmqc** for non-gradient version

  The macro will prompt you with questions about the location (experiment number) of your 1D proton and carbon experiments. Respond with the number only.

    j - average scalar coupling constant between protons and heteronucleus (one-bond constants)

    =140 for ^{13}C, **90** for ^{15}N

  For presaturation (non-gradient version):

    Set **null=0**, **nt=1**, and **dm='nnn'** and collect a 1D spectrum.

    Determine saturation frequency by setting the cursor on the peak to be saturated in your 1D spectrum, type **nl**, and **movetof**. Set the **satfrq** parameter in the hmqc equal to this value of tof.

    Set **satflg='yn'** and **satdly=1**

    satdly = length of saturation time during relaxation period (immediately after d1). Set to a value significant compared with T_1 of the peaks (~1sec)

    Array **satpwr** (power level for presaturation using transmitter) to determine the minimum value that will still remove the peak.

    Reset **null**, and set **satpwr** to the determined value.

- hmbc (uses **ghmqc** macro) -- heteronuclear multiple bond correlation (gradient version)/ **hmqc** for non-gradient version

  The macro will prompt you with questions about the location (experiment number) of your 1D proton and carbon experiments. Respond with the number only.

    mbond='y' for a multiple-bond HMQC

    null=0

    dm='nnn'

    taumb=0.055

    nt – use 4x as many scans as for the corresponding hmqc for equivalent signal-to-noise

- **ghsqc**--heteronuclear single quantum correlation, proton detected

  The macro will prompt you with questions about the location (experiment number) of your 1D proton and carbon experiments. Respond with the number only.

  This experiment is sensitive to temperature fluctuations, so set the temperature to ~5 deg above room temperature and type **su**.

    For processing, use **wft2d('t2dc', 0,1,1,0,1,0,0,1)**

J-RESOLVED EXPERIMENTS
- **hom2dj**--homonuclear 2D J experiment
  nt = 2 (minimum); 16 (recommended)
  p1=180º pulse (2*pw)
  Process with **wft2d rotate(45.0) foldj**
- **het2dj**--heteronuclear 2D J experiment
  Process with **wft2d rotate(45.0) foldj**

11. Change the parameters common to all experiments.

   Adjust **nt** and **ni** to fill up the available experiment time. Check length of experiment by typing **time**. Make sure the 90° pulse is set to the value you determined in step 6.

   pw - 90 degree pulse (from step 6) except for TOCSY or ROESY experiments
   nt - the number of scans. Increasing this number will improve signal-to-noise.
        = multiple of 4 or 8 for non-gradient experiments (due to phase-cycling)
        = 1 for gradient experiments if S/N in 1D spectrum is sufficient
   ni - the number of increments (the number of points in t_1). **ni** can be increased for better resolution.
        Do not exceed 1024.
   sw - spectral width in the directly detected dimension (F_2) (usually H1)
   sw1 - spectral width in the indirectly detected dimension (F_1) (usually C13)
   np - the number of points in each FID (t_2). Do not exceed 2048.
   d1 - usually set to 3 * T_1 (1-10s)
   phase=0 for absolute value experiments, **phase=1,2** for States-Haberkorn (hypercomplex) phase-cycling, and **phase=3** for TPPI phase-cycling. For **phase=3**, **sw1** must be set to twice **sw**.

12. Regulate the Temperature (optional)

   Regulate the temperature for long (e.g., overnight) experiments, or experiments that heat up the sample (ghmqc, ghmbc): **temp=23** then **su**.
   Remember to turn the variable temperature controller off when you are finished: **temp='n'** then **su**.
   Also, every time you move between experiments and/or call up new experimental parameters, you may need to reset the temperature and type su.

13. Starting the acquisition
   Type **go** to start the experiment. This will acquire data without processing it.

14. Check progress of the experiment
- Type **wft(1)** after the first FID is finished.
  If you do not see any peaks, perhaps you have not set up some of the parameters correctly. Type **aa** to abort the experiment and adjust necessary parameters, then type **go** again. To view other fids as they are collecting, type **wft(#)** where # is the fid that you would like to see.
- If you get any "ADC Overflow" or "Receiver Overflow" (red overflow light) errors, you should reduce the value of **gain** until you no longer get these errors.
- Conversely, if you feel the signal-to-noise should be larger than what it is, increase the value of gain. Type **gain?** and the current value of **gain** will be displayed. Increase **gain** and repeat until the error message "ADC Overflow" is seen, and then reduce gain by 2 until the message is no longer displayed. Proper adjustment of **gain** can make a big difference in the final signal-to-noise ratio of a 2D experiment!

15. Save the data
   Once the experiment is complete, save your data using the menu buttons: **[Main Menu]** - **[File]** - **[Data]** - **[Save FID]** or by typing **svf**, then a filename when prompted.

16. Turn off temperature control
   Once your data is saved, turn off the temperature control: **temp='n' su**. Insert the dummy, lock and shim.

AUTOMATIC PROCESSING

For quick processing, use the macros **wft2d** (for absolute value data) and **wft2da** (for phase-sensitive data). **foldt** to symmetrize cosy datasets. **Rotate(45)** and **foldj** for 2DJ experiments.

MANUAL PROCESSING

ZERO-FILLING

The size of the Fourier Transform is **fn**. This must have a value that is a power of 2 and determines how much zero-filling is done at the end of the FID prior to the Fourier Transform. The amount of zero-filling determines how well defined the peaks in the spectrum are. For symmetrical datasets, set **fn** equal to **fn1** or you will not be able to symmetrize your data at the end. Typical values for **fn** are 1024 or 2048 for a dataset of manageable size.

APODIZATION (WEIGHTED FOURIER TRANSFORM)

In the majority of cases, the macros **wft2d** or **wft2da** are sufficient to process the 2D data. However, there will be some instances where you will want to reprocess your data, e.g., to determine if a particular crosspeak is "real" or to see if you can get increased resolution or signal-to-noise. Apodization ("to cut off the feet") is the process of multiplying the time-domain data by a weighting function prior to Fourier Transform of the data. The effect of the multiplication is to modify the lineshape of peaks in the spectrum. Emphasis on the first part of the fid gives you sensitivity enhancement (at the expense of resolution), emphasis on the latter part of the fid gives resolution enhancement (at the expense of signal-to-noise).

1. For manual processing, first type **wft(1)** to see the first spectrum of your dataset. Phase the spectrum. Set **fn=fn1** (see above) for a symmetrical dataset if necessary. Set **pmode='full'** if this is a phase-sensitive spectrum.

2. Integrate, and baseline correct (**bc**) the spectrum. In order to do baseline correction, *all* peaks in your spectrum must be integrated.

3. The weighting function can be customized using the interactive weighting program by typing **wti** or using the menu buttons **[Main Menu]** - **[Process]** - **[Adj Weighting]**.

This will display the spectrum on the top, the FID on the bottom, and the weighting function in the center. The vertical scale of the FID and spectrum can be adjusted using the middle mouse button; the value of the weighting function parameter can be adjusted using the left mouse button. Which weighting functions are used are chosen by selecting the menu buttons that appear with the **wti** display.

> The weighting functions are: line broadening (**lb**), sine-bell (**sb**), shifted sine-bell (**sbs**), Gaussian (**gf**), shifted Gaussian (**gfs**), or additive weighting constant (**awc**). The shifted functions work together with their corresponding function, e.g., first find an appropriate sinebell with **sb** before trying to shift it with **sbs**.

If you want to type in values for an apodization function, e.g., **sb=0.01**, you must select **[Adj Weighting]** in the menu bar to see that function applied to the spectrum. The current values are displayed at the bottom of the screen.

Absolute value data is usually processed with sinebell weighting. Adjust the sinebell so that the data decays to zero before the end of the FID. The sinebell can be made negative by clicking on the outside of the apodization window on the left side.

Phase-sensitive data is generally processed with Gaussian weighting. Adjust Gaussian weighting so that the data decays to zero before the end of the FID.

4. After choosing the correct weighting function, select **[return] - [Transform F2]** or type the appropriate processing macro as listed after each experiment, e.g., **wft1da** for a DQCOSY, **wft1d(1,0,0,1,0,1,1,0)** for a phase-sensitive gradient NOESY. This will process the F_2 dimension.

5. Choose a **[trace]** through one of the interferograms by clicking on **[trace]** and moving the cursor to one of the interferograms displayed.

6. Choose **[Return] - [Adj Weighting]** to adjust the weighting in the second dimension or type **wti** and choose an appropriate apodization function.

7. Process the second dimension by clicking on **[return] - [Transform F1]** or with the appropriate transform macro, e.g., **wft2da** for DQCOSY or **wft2d(1,0,0,1,0,1,1,0)** for a phase-sensitive gradient NOESY. This will complete the transformation of the F_1 dimension.

8. For COSYs and other symmetrical datasets, i.e, homonuclear datasets, you may want to symmetrize your dataset:

   foldt or with the menus: **[Main Menu] - [Display] - [Massage] - [foldt]**

   Note that this is only possible if **fn=fn1**. You must set these values before processing.

9. If you integrated your peaks, you can execute baseline correction in both dimensions by typing **bc('f1')** and **bc('f2')**. In some cases, this may get rid of some t1 noise.

PHASING

For phase sensitive data (e.g., NOESY), it is often necessary to do a final phasing of your peaks.

1. You must set **pmode='full'** to be able to phase in both dimensions.
2. Click on **[trace]** and select a trace in the upper right section of the 2D spectrum.
     Note the index number of this trace.
3. Enter **ds(#)** to display the trace and phase as you would a 1D spectrum**.**
     This adjusts the **rp1** value. Take note of the ppm value of this peak.
4. **dconi** to redisplay with the new **rp1** value
5. Click on **[trace]** again and select a trace in the lefthand section of the spectrum.
6. Enter **ds(#)** and click on **[phase]**.
7. Move the cursor *upfield* (to the position of the *first* peak that you phased) and click.
     Do not adjust the phase at this point. Clicking here sets the previous rp1 value.
8. Move downfield (to the peak) and click. Adjust the phase normally. This adjusts **lp1**.
9. **dconi** to display the properly phased 2D spectrum. Repeat steps 2-8 as necessary.
10. To phase in the other dimension, type **trace='f2' dconi** and repeat the phasing process. To return to the original trace, type **trace='f1'**.
11. Baseline correct (if you have integrated your peaks previously) by **bc('f1')** and **bc('f2')**.

DISPLAYING THE SPECTRUM

To view your processed data, type **dconi**.

To expand regions, use the left and right mouse buttons to form a box around the region of interest and click on **[expand]**. The left cursor should be in the lower left region and the right cursor in the upper right region.

vs2d =# adjusts the vertical scaling or use the **[vs+20%]** and **[vs-20%]** buttons.
The middle key of the mouse adjusts the threshold level using the sliding color scale on the right-hand side of the spectrum. Use this in conjunction with the **vs2d** command to find the appropriate contour level for a minimum amount of noise.

dpcon--displays contoured spectrum (not interactive)
This command requires arguments for the number of contours and the spacing between them, e.g., **dpcon(10, 1.2)** for 10 contours spaced 1.2 levels apart. Adjust these values to see how the levels will look when plotted. For phase-sensitive data, the arguments **'pos'** and **'neg'** can be used to display only the negative or positive peaks, e.g., **dpcon('pos',10,1.2)**.

dpconi--displays contoured spectrum in interactive mode (although your interaction will be slow). See above.

To display 1D projections: **[proj] - [Hprojmax]** and **[proj] - [Vprojmax].** Use the middle mouse key to adjust peak intensity of these projections.

To control the size of the plot, there are several parameters. Always redisplay your data with **dconi** to see the effect of any changes.

sc is the start of the plot with respect to the right side of the page (in millimeters) in the x dimension.
wc is the width of the plot (in millimeters) in the x dimension.
sc2 is the start of the plot with respect to the bottom edge of the page (in millimeters) in the y dimension.
wc2 is the width of the plot in millimeters in the y dimension.

sp is the start of the plot in Hz (from the upfield side) in the directly detected dimension.
wp is the width of the plot in Hz in the directly detected dimension.
sp1 is the start of the plot in Hz (from the upfield side) in the indirectly detected dimension.
wp1 is the width of the plot in Hz in the indirectly detected dimension.
   These can be converted to ppm by using a **p** after the value, e.g., **sp=4p**

To plot symmetical homonuclear datasets, you will want **sp=sp1** and **wp=wp1.** For square plots, use **wc=wc2.**
These are not so important, nor necessarily desired, for heteronuclear experiments.

REFERENCING

For symmetrical homonuclear datasets, place the cursor on the peak to be referenced.
   rl(#p) in the first dimension (f1) sets the reference line in ppm. Alternatively, type **rl(#*sfrq)**.
   rl1(#p) in the second dimension (f2) sets the reference in ppm. Alternatively, type **rl1(#*sfrq)**.
Referencing can also be done by setting **rfl=rfl1** and **rfp=rfp1**. The values of **rfl** and **rfp** are conveniently obtained
from a properly referenced 1D spectrum *that has the same sweepwidth* as your 2D spectrum.

For heteronuclear datasets, **rl(#p)** or **rl(#*sfrq)** will set the directly detected dimension. **rl1(#d)** or **rl1(#*dfrq)** will
set the reference in the indirect dimension.

PLOTTING
NEVER plot your 2D dataset while it is still acquiring or you may lose it entirely.

Method 1: using macros

1. Load your 1D spectrum with the reduced sweepwidth into experiment 1 (**jexp1**).   Process and phase it.
2. If this is a heteronuclear experiment, load the carbon spectrum into experiment 2 (**jexp2**).
3. Go to another experiment and load and process the 2D experiment, e.g., **jexp3**.
4. For homonuclear experiments (eg, cosy, noesy):
   plcosy(10,1.2,1) plots a 2D dataset with 10 contours spaced 1.2 levels apart and plots the 1D dataset from
   experiment 1. The macro **plcosy** takes 3 arguments: number of contours, spacing of levels, and experiment
   number that contains the 1D data. Do not type **page**.
5. For heteronuclear experiments (eg., ghmqc, ghmbc):
   plhxcor(10,1.2,1,2) to plot a heteronuclear data. This command can have up to 4 arguments: number of
   contours, spacing of levels, experiment number for proton experiment, experiment number for carbon
   experiment. Do not type **page**.

Method 2: manual plotting if you have a 1D spectrum of the same spectral width

For manual plotting, use the **pcon** command. This is analogous to the **pl** command for 1D spectra.
1. Load and process the 2D spectrum. The **pcon** command uses arguments similar to **dpcon**,
   e.g., **pcon(10,1.2)** plots 10 contours, spaced 1.2 levels apart.
2. Type **ppa** if you want parameters.
3. Load the appropriate 1D spectrum into another experiment and process it.
4. From the 1D experiment, type **partop** if you want the spectrum plotted on the top
5. Answer the question about what experiment the 2D spectrum is in. Use the middle mouse button to adjust the
height of the projections.
6. Type **pl('top')**
7. From the 1D experiment, type **parside** if you want the spectrum plotted along the side
8. Answer the question about what experiment the 2D spectrum is in. Use the middle mouse button to adjust the
height of the projections.
9. Type **pl('side')**

10. Type **page**.

Method 3: manual plotting if you do NOT have a 1D spectrum of the same spectral width

For manual plotting, use the **pcon** command. This is analogous to the **pl** command for 1D spectra.
1. Load and process the 2D experiment. The **pcon** command uses arguments similar to **dpcon**,
     e.g., **pcon(10,1.2)** plots 10 contours, spaced 1.2 levels apart.
2. Type **ppa** if you want parameters.
3. Select **[proj] - [Hprojmax] - [plot]**
     You may need to use the middle mouse button to adjust the height of the projections.
4. Select **[Vprojmax] - [plot]**
5. Type **page**.