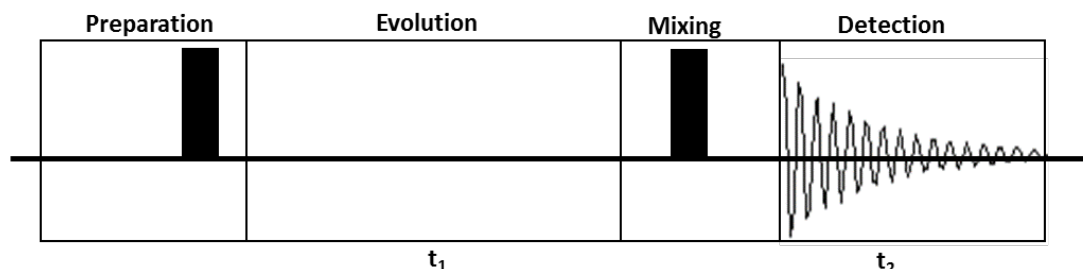


JEOL ECS 400 - 2D NMR Measurements Set-up and Protocol

1. Introduction: How does 2D NMR work?

The picture below shows a basic 2D sequence (in this example, COSY experiment with two 90-degree pulses).



2D spectra are acquired “point-by-point” in t_1 (*evolution time*). This means that a series of FIDs is collected, one for each value of t_1 , which is incremented systematically by $1/(\mathbf{y_sweep} \text{ [Hz]})$, starting from $t_1=0$. Finally, a data matrix is obtained, in which we can picture the rows as representing actually acquired FIDs (during *detection* period t_2). Since each row represents a different value of evolution time, columns give the intensity of the signal as a function of t_1 . After Fourier transformation (**FT**) of the rows, each column shows the intensity of a particular signal in F_2 (*directly observed dimension*) as a function of t_1 . Then, we can perform FT of those columns and convert the columns into the second frequency dimension, called F_1 (*indirectly observed dimension*).

In practice, when setting up 2D experiment, we need to set **y_sweep** (the range of frequencies to be indirectly observed) and choose the number of FIDs (increments), called in Delta software **y_points**. The number of increments which is related to the total number of FIDs that will be acquired, and therefore to total experiment time, depends on the resolution required in F_1 . To achieve higher F_1 resolution, larger values of **y_points** are required but this may result in significantly longer experiment time. This time can also be used for getting better signal to noise ratio (**S/N**) in each increment by increasing number of scans per increment (**scans**). In homonuclear 2D experiments one needs to set only the **x_offset** and **x_sweep** because corresponding y values are identical.

2. General hints for 2D Experiments:

- Minimize the spectral window in both dimensions. This results in the highest F_1 resolution for a given number of increments and produces smallest data sets. It also minimizes off-resonance effects.
- Optimize spectrometer conditions (spectrometer stability):
 - Turn off sample spinning. The higher resolution achievable with sample spinning is not needed in 2D spectra and sample spinning can never be ideally stabilized.
 - Regulate the sample temperature and (if possible) room temperature.
 - Optimize lock conditions. Lock *Level* just below saturation, lock *Gain* low (after shimming).
- Do not acquire more increments or scans per increment than are required

S/N for each increment spectrum needs to be only about 5:1

Remember square root factor for increasing S/N with increasing *scans*. In partitioning total experiment time between scans per increment and number of increments it seems to be better to emphasize S/N of the increments more heavily than F1 resolution.





The following instructions assume reader's familiarity with Delta software and ability to set up basic 1D proton and 13C experiments. These are rather simple instructions (hopefully, not oversimplified). Consulting Delta/ECS manuals and watching excellent Delta Tutorial videos is highly recommended.





3. COSY (COrrelation SpectroscopY)

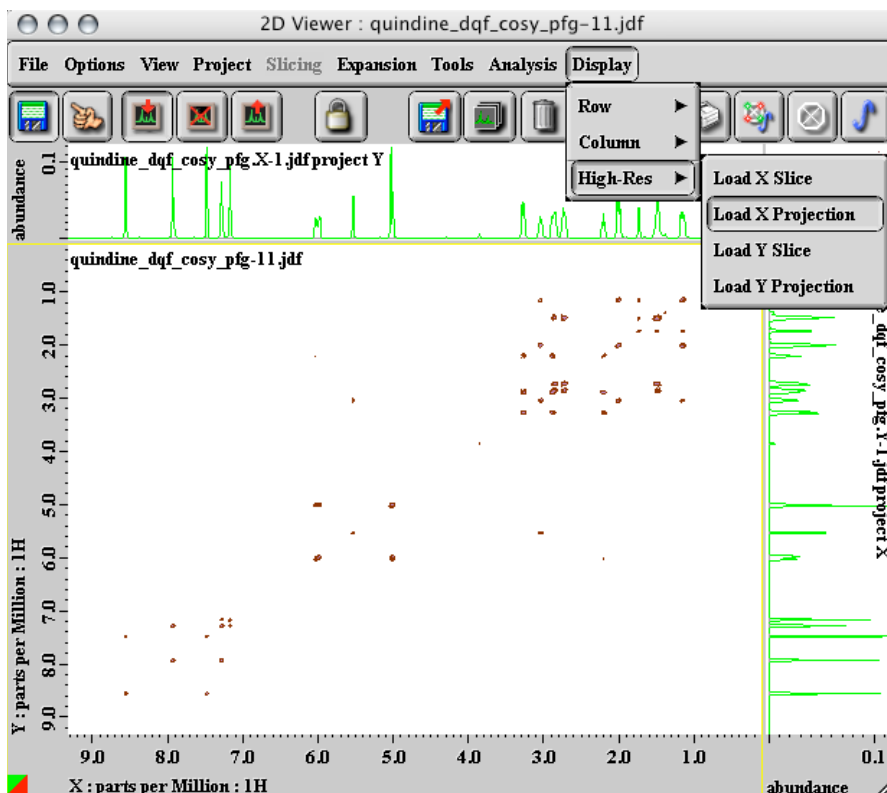
This is an example of a homonuclear 2D correlation experiment (like TOCSY or NOESY). COSY experiment is run to assign proton connectivity. Sometimes **DQF-COSY** (Double Quantum Filtered) is preferred for suppression of the strong singlets which allows one to set higher receiver gain than that used in its corresponding proton experiment. However, DQF-COSY attenuates all signals to some extent which may cause problems with observation of weak cross-peaks. Except for a different pulse sequence name (dqf_cosy_pfg.ex2) the setup is basically the same for both experiments.


3.1 COSY setup and processing

Run a 1D proton spectrum. Note the gain value determined for the proton spectrum. As a first guess, use this receiver gain in the COSY experiment. Zoom the peaks in the proton data so there is about 0.5 ppm on either side of the peaks and leave the processor window open.

- 3.2 In the **Spectrometer Control** window, click on the **Expmnt** button. Click on the  button and select *cosy_pfg.ex2* experiment.
- 3.3 In the **Experiment Tool** window, click on the **Instrument** tab and set **recvr_gain** to the value just used for the 1D proton. **Spectrometer Control** can display the current receiver gain (**RG**) value – select **Options->Monitor Params. Delta Console** also reports receiver gain values.
- 3.4 Switch to the **Experiment Tool / Acquisition** tab and set number of **scans**. It is set by default to 1 because the pfg COSY can be run with one scan per increment (the non-gradient version requires at least 4 scans). Of course, you can set scans to a higher number if you need higher signal to noise ratio of the cross-peaks.
- 3.5 In the same **Acquisition** tab, click on  and then on the  button. Next, click on the zoomed proton spectrum. The **x_offset** and **x_sweep** will be now set to the values reflecting the zoomed data set. If you don't see the  icon, select **View->View Buttons**.
- 3.6 You can also adjust **x_points** and **y_points** from the Acquisition tab. These parameters determine the x and y (F_2 and F_1) resolution. The default values are suitable for most small molecules. Remember, that increasing **y_points** (in order to improve F_1 resolution) may dramatically increase **Total Collection Time**, shown at the bottom of the **Experiment Tool** window.

- 3.7 Turn off the spinner by clicking on the  button in the **Sample** window and in the **Experiment Tool** window click on . When data acquisition has been completed, the auto-processed 2D COSY spectrum is displayed in the **nD Processor** window. If you do not need to make process list changes, send spectrum to **2D Viewer** by clicking on the  button. Otherwise, proceed to 3.11.
- 3.8 After clicking on the  button you should see something like that:





Notice that there are low-resolution 1D projections drawn along the x and y axis of this plot. These projections are generated from the intensity dimension of the 2D data. It is convenient to load high-resolution proton spectra into these fields. Click  in the **2D Viewer** to toggle operation from disk to finger mode. Select **Display**→**High-Res**→**Load X Projection** (as shown on the picture above) and then click on the processed proton spectrum. This will load the proton data set into the x-axis of the viewer. Repeat the procedure using **Load Y Projection** for the y axis. The 1D and 2D data are now connected. Therefore, any zooming done in either 1D or 2D portions of the window will be synchronized.

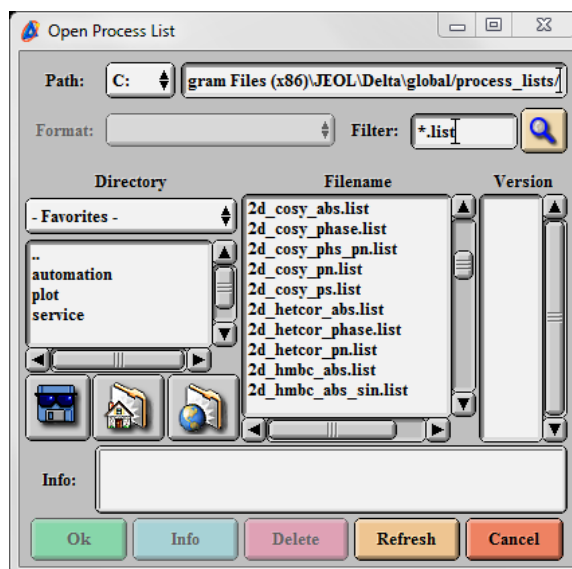
- 3.9 Next step, after loading the 1D projections, would be contouring adjustment using the **Level Tool**. It can be opened by right-mouse-hold on the spectrum area and selecting **Level Tool** (keyboard shortcut is the / key). Usually, it is only necessary to turn off only one or two of the bottom contours to get a clear 2D “map”. Detailed instruction for using this tool is

beyond the scope of this short tutorial but **Level Tool** operation is well explained in one of the *Delta Tutorials* videos.

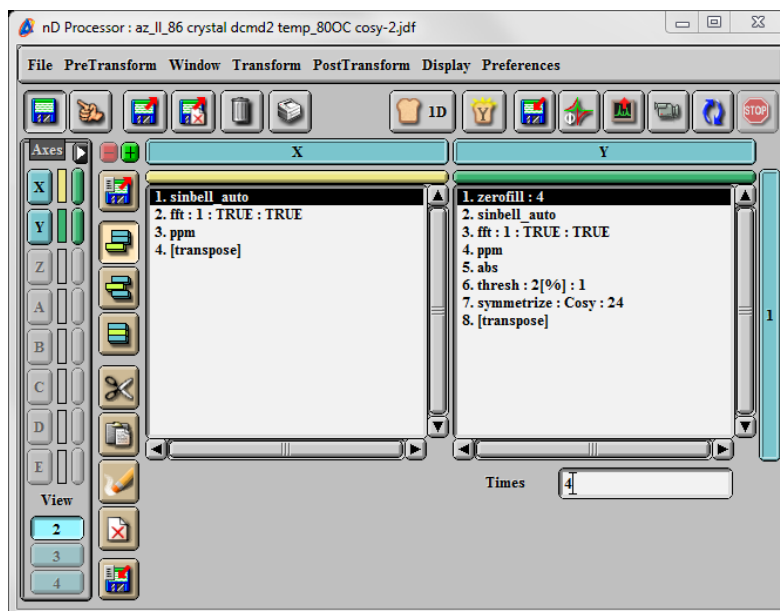
3.10 If it is necessary to modify the processing parameters after the COSY spectrum is



displayed in the **nD Processor** window (as in 3.7), you need to click on . This opens two processing lists for both x and y dimensions. Click on a list to select it if you wish to modify any of its items. It is possible to make a new list or add items to existing ones by choosing

them from the menus. You can also load a processing lists from disk by clicking on . The **Open Process List** will be displayed, as shown below:



3.11 The default COSY process list is matched to the default acquisition parameters:




If you changed the number of **y_points**, you will need to modify **zerofill**. The default COSY parameters specify 1024x256 data matrix size but COSY axes should be symmetric. Therefore, in order to make the matrix symmetric 1024x1024, we need to apply a **zerofill** value of 4. If for example, **y_points** has been set to 512, we will need a **zerofill** value of 2. The other processing parameters can be selected and modified as well and the effects can be viewed by hitting the  button. After that, clicking on  sends the processed data to the **2D Viewer**.

4. HMQC (Heteronuclear Multiple Quantum Coherence)

This is an example of a heteronuclear 2D correlation experiment (like HETCOR, HSQC, or HMBC). It is used to assign proton carbon connectivity. Unlike HETCOR, it is a proton-detected experiment because it detects more sensitive ^1H nucleus instead of ^{13}C . This experiment is particularly useful if you are sample-limited since it will require fewer scans per increment than HETCOR (which is ^{13}C -detected experiment). However, resolution obtained in the indirectly-observed ^{13}C dimension is of course lower than in HETCOR. Because the experiment filters out 12C-1H peaks, the receiver gain should be set higher than that used to collect corresponding proton spectrum to take advantage of the inverse detection.




4.1 HMQC setup and processing



Setting up of the HMQC acquisition is quite similar to setting up homonuclear correlation like COSY. First, acquire proton and ^{13}C (if possible in reasonable time) spectra. Zoom the peaks in the proton data so there is about 0.5 ppm on either side of the peaks and leave the processor window open. Do the same with the ^{13}C data (leaving about 5 ppm on either side of the peaks) and leave the window on the desktop.

4.2 In the **Spectrometer Control** window, click on the **Expmnt** button. Click on the  button and select *hmqc* → *hmqc_pfg.ex2* experiment.








4.3 In the **Experiment Tool** window, click on the **Instrument** tab and set **recvr_gain** to at least the value just used for the 1D proton. You can also try to set it much higher than that and check if Delta reports receiver overflow during data acquisition.

4.4 Switch to the **Experiment Tool / Acquisition** tab and set number of **scans**. This value of course, depends on the sample amount.

4.5 In the same **Acquisition** tab, click on  and then on the  button. Next, click on the zoomed proton spectrum. The **x_offset** and **x_sweep** will be now set to the values reflecting the zoomed proton data set. If you don't see the  icon, select **View->View Buttons**.

4.6 Click on  and then on the  button. Next, click on the zoomed ^{13}C spectrum. This will set the values of **y_offset** and **y_sweep**.

4.7 You can also adjust **x_points** and **y_points** from the Acquisition tab. These parameters determine the x and y (F_2 and F_1) resolution. The default values are suitable for most small molecules but you may need more points if **y_sweep** value is particularly large and you need

- (relatively) good resolution in ^{13}C dimension. Now check **Total Collection Time**, shown at the bottom of the **Experiment Tool** window.
- 4.8 Turn off the spinner by clicking on the  button in the **Sample** window and then in the **Experiment Tool** window click on . When data acquisition has been completed, the auto-processed 2D HMQC spectrum is displayed in the **nD Processor** window.
- 4.9 Send spectrum to **2D Viewer** by clicking on the  button. Click  to toggle operation from disk to finger mode. Select **Display**→**High-Res**→**Load X Projection** (as shown on the picture above) and then click on the processed proton spectrum. This will load the proton data set into the x-axis of the viewer. Repeat the procedure using **Load Y Projection** for the y axis but this time click on the 1D ^{13}C spectrum. Contour the data, as needed, using the **Level Tool** as described for COSY processing.
- 4.10 As described for COSY, if it is necessary to modify the processing parameters after the HMQC spectrum is displayed in the **nD Processor**, you need to click on . This opens two processing lists for both x and y dimensions. Click on a list to select it if you wish to modify any of its items. The effects of the parameters adjustment can be viewed by hitting the  button. After that, clicking on  sends the processed data to the 2D Viewer.