5   Basic Procedures

This chapter will outline basic operations which are used every time a spectrum is acquired. The TopSpin software has an easy to use work flow-based interface which guides a user through all the necessary steps to obtain a spectrum. All operations are software controlled and can be selected with a mouse click on the work flow tabs (see 12. in the TopSpin Window figure), which opens up a row of work flow buttons (see 11. in the TopSpin Window figure).

The readers may skip this chapter if they are already familiar with these operations.

5.1   The TopSpin Window

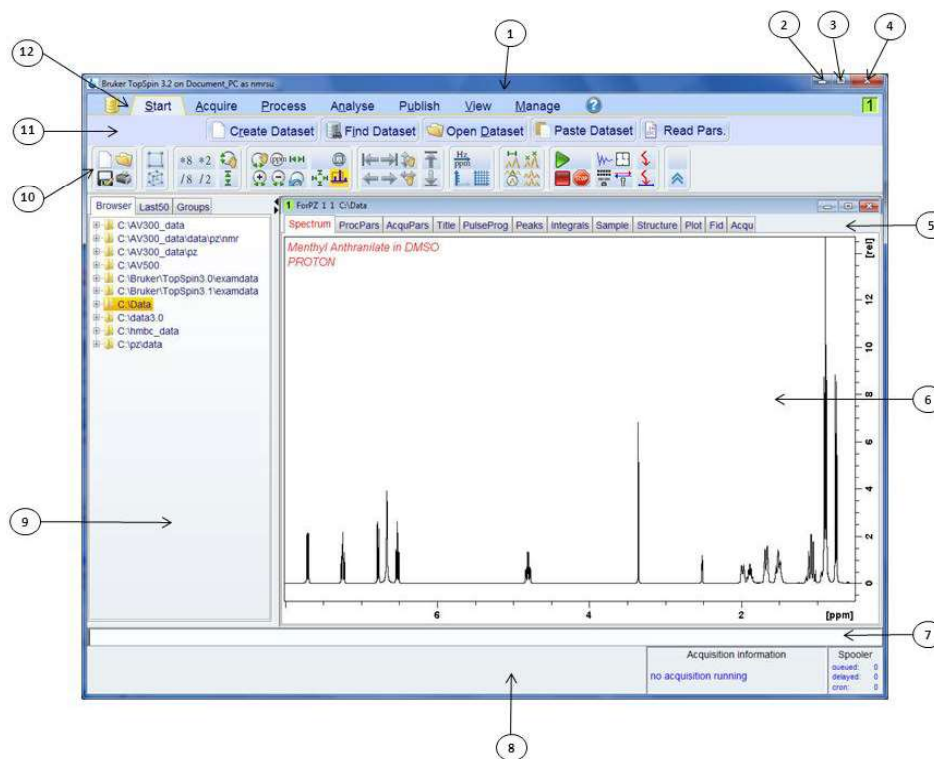The picture below shows the layout of the basic TopSpin window.

Figure 5.1: The TopSpin Window

| 1.	Title bar	7.	Command line
2.	Minimize button	8.	Status bar
3.	Maximize button	9.	Browser window
4.	Close button	10.	Tool bar
5.	Data Set tabs	11.	Workflow button bar
6..	Data Display window	12.	Workflow menu bar

5.1.1 Creating a New Data Set

Regular use of a spectrometer will soon result in the accumulation of large amounts of data. Users will want to store data in conveniently named files so that they can easily access their data later. This is particularly important in a multi-user environment. When a set of data is acquired, it can be stored in what is called a **data set**. Each data set must have a unique descriptor (identifier) so that different data sets can be distinguished. A full descriptor for a data set requires the use of four parameters: DIR, NAME, EXPNO, and PROCNO.

1. Click on the **Start** tab in the Workflow tab bar.
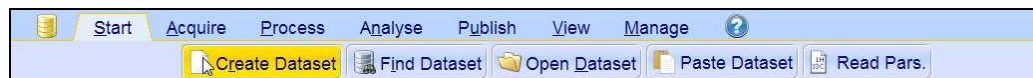
2. Select the **Create Dataset** button in the Workflow button bar by clicking on it.
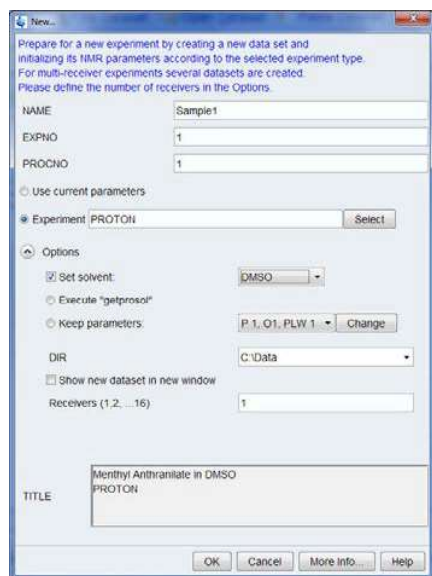
3. You can now create your own data set. For NAME you may enter a string of up to 13 characters. For both EXPNO and PROCNO enter '1'.

4. Choose the experiment you want to run by clicking on the **Select** button to the right of the Experiment window.

5. Enable **Show Recommended** inside the experiment selection window.
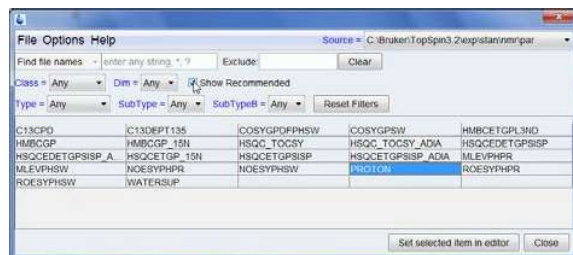
6. Select the experiment (e.g. Proton).

7. Click on the **Set selected item in editor** inside the experiment selection window.

8. Choose the solvent of your sample by clicking on the **down arrow** to the right of the Solvent window.

9. Select the solvent (e.g. DMSO) by clicking on it.

10. Choose a directory path where your data set is being stored on the computer's hard drive by clicking on the **down arrow** to the right of the DIR window.
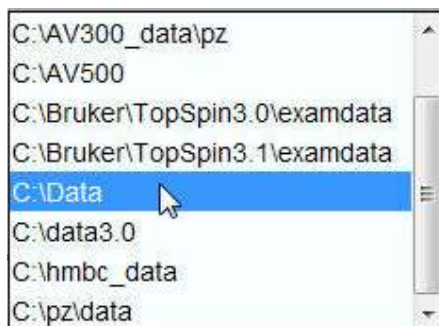
11. Select the directory path (e.g. C:\Data)

12. Enter a desrciption of your sample in to the **Title** window.

13. Click on the **OK** button inside the New Data set window.

Note: Your newly created data set is now the current data set and details of the descriptor will be stored on the harddrive in the folder: DIR\NAME\EXPNO, where each EXPNO corresponds to a completely independent data set. In this example, the data will be stored at **C:\Data\Sample1\1**. This path will also appear in the title bar of the data window of Topspin

---

5.1.2   Sample Preparation

- Use clean and dry sample tubes.
- Use medium to high quality sample tubes.
- Always filter the sample solution.
- Always use the same sample volume or solution height.
- Filling volume of a 5 mm tubes is 0.6 ml or 5 cm.
- Filling volume of a 10 mm tubes is 4 ml or 5 cm.
- Use the sample depth gauge to adjust the sample depth (1.8 cm for older style probes, 2.0 cm for newer style probes).
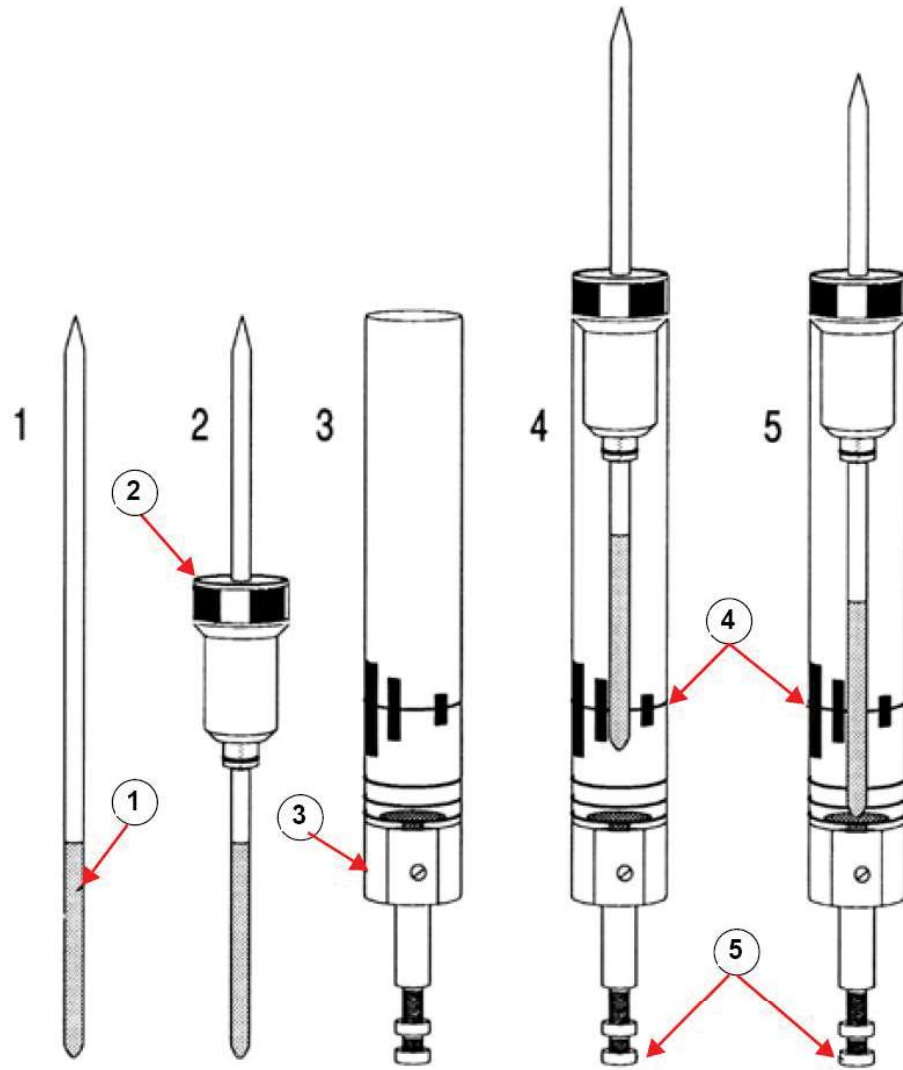
Inserting the Sample into the Spinner

1.	Sample	4.	Center Line
2.	Spinner	5.	Depth Adjustment Screw
3.	Depth Gauge		

- The sample tube should sit tightly inside the spinner.
- Wipe the sample tube clean before inserting into magnet.

5.2 Inserting the Sample plus Spinner into the Magnet

The raising and lowering of the sample is controlled by a stream of pressurized air. Before placing a sample onto the top of the bore, make sure that the air flow is present (it is quite audible) and remove any sample which may be rising to the top of the bore.
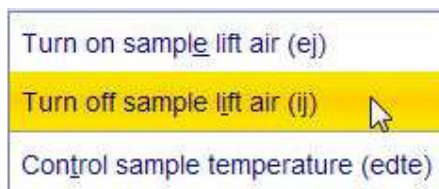
1. Click on the **Acquire** tab in the Workflow tab bar.
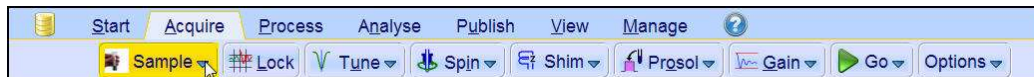
2. Click on the **down arrow** inside the **Sample** button in the Workflow button bar.


3. Select **Turn on sample lift air (ej)** by clicking on it.

4. Place the sample plus the spinner on to the top of the magnet bore.

5. Click on the **down arrow** inside the **Sample** button in the Workflow button bar.


6. Select **Turn off sample lift air (ij)** by clicking on it.

5.3 Locking the Sample

Deuterated solvents are used to generate the signal to be detected and monitored by the lock system. The frequency and strength of this signal will depend on the solvent used. The main feature of the TopSpin lock routine is that it sets parameters such as the lock power, gain and frequency to a value appropriate to the solvent. With these default values set close to that which would be expected for that solvent, the BSMS can quickly locate and "lock" onto the solvent signal by sweeping through a range of frequencies or magnetic field values. The solvent dependent parameters are taken from the "edlock" table.
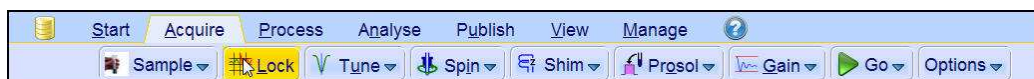
1. Select the **Lock** button in the Workflow button bar by clicking on it.
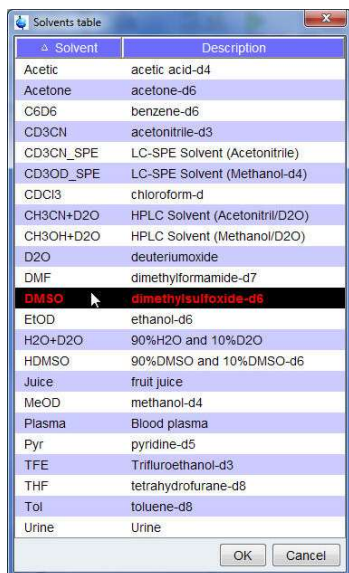
2. Select a solvent by clicking on it.

3. Click on the **OK** button inside the Solvent table window.

ℹ️ **Note**: If lock has been achieved, the signal should be of the form of a horizontal line with some associated noise or ripples (see figure below). The height of this line is called the lock level.
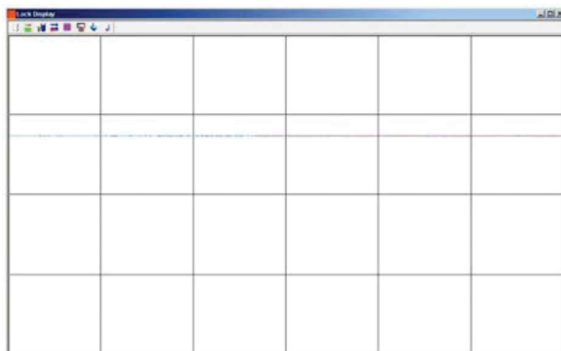

Figure 5.2: Lock Display after Locking the Sample

5.4 Tuning and Matching the Probe

The sensitivity of any probe will vary with the frequency of the signal transmitted to it and there exists a frequency at which the probe is most sensitive. Furthermore this frequency may be adjusted over a certain range using tuning capacitors built into the probe circuitry. **Tuning** involves adjusting the probe circuitry so that the frequency at which it is most sensitive is the relevant transmission frequency (SFO1, SFO2 etc.) Each coil in the probe will be tuned (and matched) separately. If the probe has been changed or the transmission frequency altered significantly, it may be necessary to retune the probe. For routine work in organic solvents with selective probes, the value of the transmitted frequencies are unlikely to vary greatly. Hence, once the probe has been initially tuned, slight variations in frequency will

not warrant retuning. Typically the transmitted frequency would need to be altered by at least 100kHz to warrant retuning. However for broadband probes the frequencies transmitted will vary greatly from nucleus to nucleus and so the probe will need to be tuned each time the selected nucleus is altered. Whenever a probe is tuned it should also be matched. **Matching** involves ensuring that the maximum amount of the power arriving at the probe base is transmitted up to the coil which lies towards the top of the probe. This ensures that the minimum amount of the power arriving at the probe base is reflected back towards the amplifiers (and consequently wasted).

Note: Bruker offers two different types of tuning and matching adjustments. In addition to the manual adjustments of the tuning and matching capacitors, the probes can be equipped with an Automatic Tuning Module (ATM). Follow the steps below for either option.

## 5.4.1 Probes Equipped with ATM using the Automated Tuning Routine

• Select the **Tune** button in the Workflow button bar by clicking on it.

The display will switch automatically to the acquisition window and displays the wobble curve. The tuning and matching is performed automatically. If multiple frequencies are used in a parameter set such as C13CPD etc., ATMA will start adjusting the lowest frequency first and will switch in the order of increasing frequency automatically.

5.4.2 Probes Equipped with ATM Using the Manual Tuning Routine
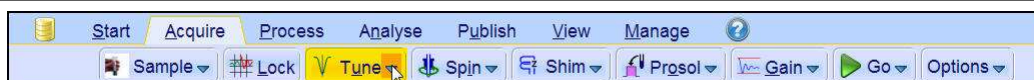
• Click on the **down arrow** inside the **Tune** button in the Workflow button bar.
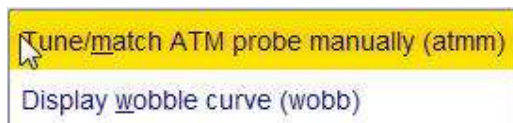
• Select **Tune/match ATM probe manually (atmm)** by clicking on it.

Note: The ATMM Probe Tuning/Matching window (figure below) and a window displaying the wobble curve will be opened.
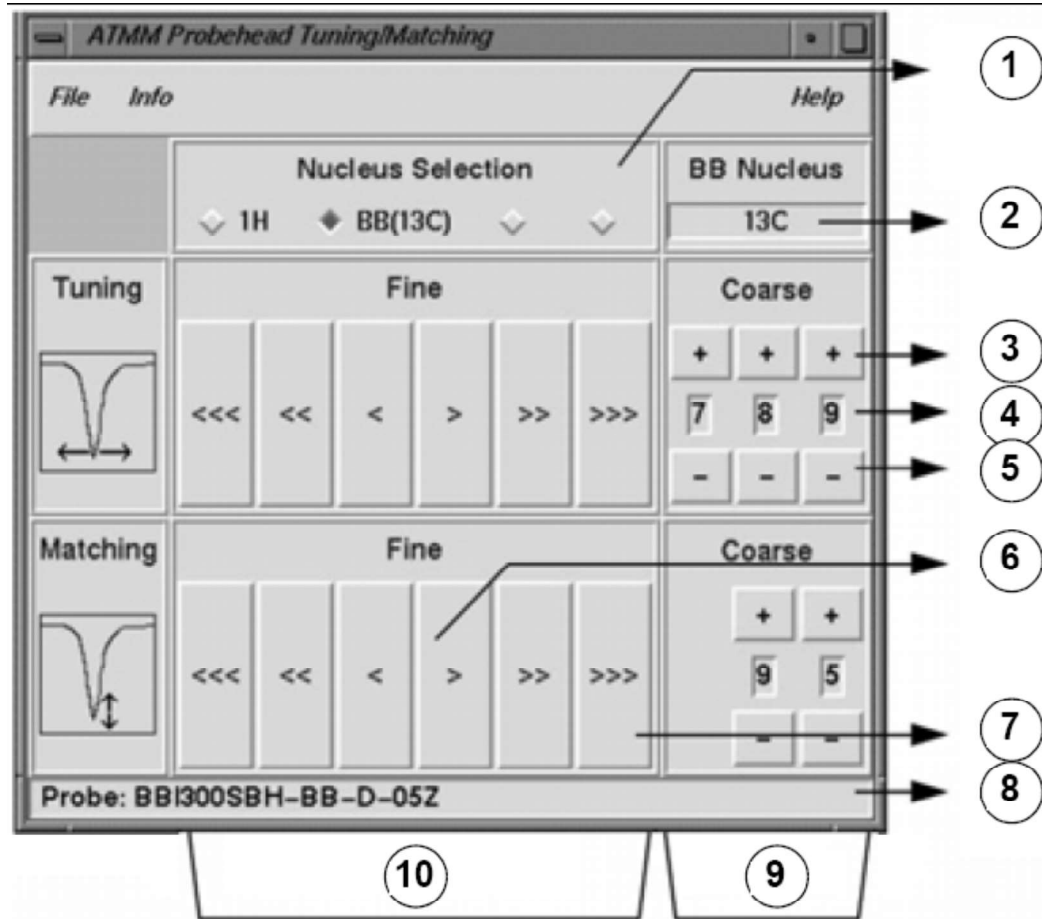
Figure 5.3: ATMM Probe Tuning/Matching Window

1.	Select next nucleus to wobble on (only routed channels are visible).
2.	Selected nucleus on broad-band channel.
3.	Move step up.
4.	Nucleus position.
5.	Move step down.
6.	Move slow (red, inactive button indicates no further change in this direction).
7.	Move fast.
8.	Probe type.
9.	Coarse tuning and matching on broadband probes.
10.	Fine tuning and matching on all probes.

Select a suitable nucleus for your experiment and then make the adjustment by clicking the buttons with the arrows. Always start with matching, then the tuning.

The **wobble** routine works by transmitting a weak signal to the probe and comparing the probe and cable impedance with a 50 Ohm reference within the HPPR. The transmitted frequency is centered on SFO1, SFO2 etc. but "swept" over a range determined by the size of the parameter WBSW (see below). The resultant curve is the familiar response curve of a resonance circuit and is simply a measure of the amplitude of the reflected signal (vertical axis) against frequency (horizontal axis).

Matching involves adjusting the probe so that the minimum of the wobble curve is at the base of the display (i.e. touching the horizontal frequency axis). This represents minimum reflection of the transmitted signal.

Tuning involves ensuring that this occurs at the frequency of transmission which will be at the center of the screen's horizontal scale. It will be seen however that the tune and match adjustments interact with each other and must be adjusted in tandem. When the wobble curve minimum is centered and at the base of the screen the probe has been optimally tuned and matched.
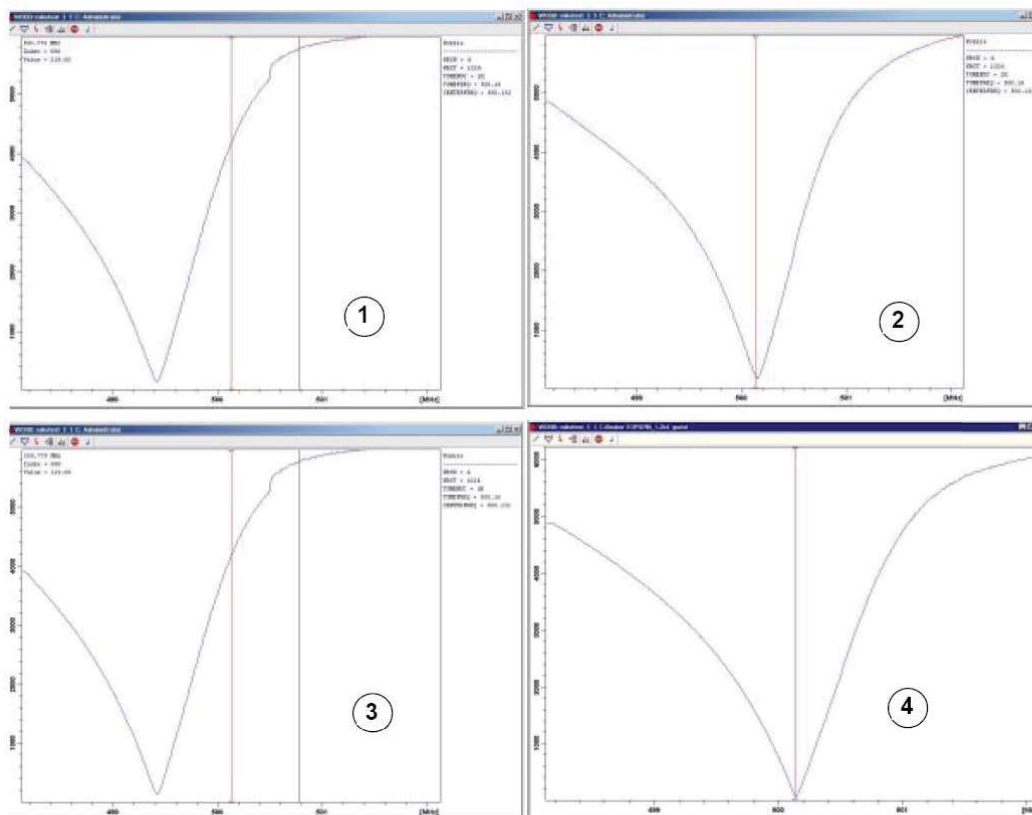

Figure 5.4: Examples of Wobble Curves with Different Tuning and Matching

1.	Bad matching and tuning.	3.	Good matching, bad tuning.
2.	Bad matching, good tuning.	4.	Good matching and tuning.

If you want to optimize the probe for several nuclei (e.g. decoupling experiments) you can choose the next nucleus in the ATMM Probe Tuning/Matching Window.

5.5 Spinning the Sample

A second function of pressurized air is to enable the sample to rotate. The spinning of the sample serves to "even-out" some of the inhomogeneity that may exist in the magnetic field at the center of the magnet.
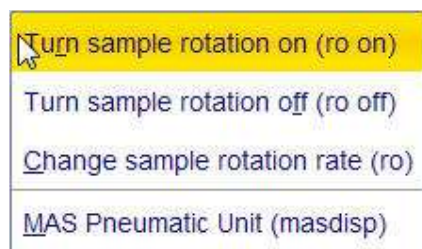
> **Note**: Samples to be investigated using experiments such as 2-D, 3-D, SELECTIVE, NOEDIFF, T1 and all inverse probes are normally not rotated.

Suggested spin rates are:
- 20 Hz for a 5 mm probe
- 12 Hz for a 10 mm probe
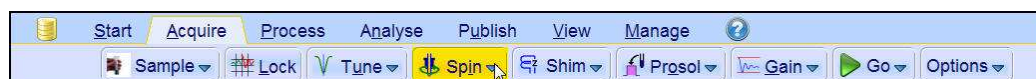
- Click on the **down arrow** inside the **Spin** button in the Workflow button bar.

- Select Turn sample rotation on (**ro on**) by clicking on it.

5.6 Shimming

Shimming is a process in which minor adjustments are made to the magnetic field until the field homogeneity (uniformity) is optimized. Improving the homogeneity will result in better spectral resolution. It will be necessary to re-shim each time a probe or sample is changed. The system manager has stored appropriate shim values (in so called shim files) for each probe that will greatly reduce the shimming time required whenever a probe is changed.

5.6.1 Routine Shimming Using TopShim

This is routine shimming and should be carried out at the beginning of every NMR session, and whenever the sample in the magnet is changed. Routine shimming involves making fine adjustments to the Z, Z^2, Z^3, Z^4 and Z^5 shims. Some higher field magnets may require higher order Z shims. The system administrator has programed TopShim to achieve the best homogeneity on each sample and it is fully automatically.

The core method of TopShim is gradient shimming. A quality criterion for the final line-shape ensures best results for all situations.

TopShim is using for all deuterated solvents the ^2H gradient shimming method and for other solvents especially H_2O, the ^1H gradient shimming method.

- Select the **Shim** button in the Workflow button bar by clicking on it. The shimming will start momentarily and should take less than a minute.

## 5.7   Setting the Probe/Solvent Dependent Parameters

Parameters, such as the 90^0 or the decoupler pulse length with associated power levels can be stored for any specific nucleus on any available channel. These values have been entered by the installation engineer or by the system administrator and are automatically loaded clicking on the **Prosol** button.

- Select the **Prosol** button in the Workflow button bar by clicking on it.

5.8   Adjusting the Receiver Gain

The receiver gain is a very important parameter that is used to match the amplitude of the FID to the dynamic range of the digitizer. The gain is automatically set clicking on the **Gain** button.
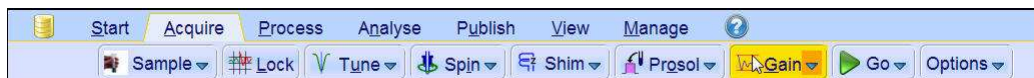
- Select the **Gain** button in the Workflow button bar by clicking on it. The receiver gain adjustment will start momentarily and it should take less than a minute.

5.9   Starting the Acquisition

The process of receiving the NMR signals is called an acquisition. Data is said to be acquired. When an acquisition is carried out, "raw" data is acquired and the received signal is called an FID (Free Induction Decay). The digital point size of the FID is called TD, Time Domain.
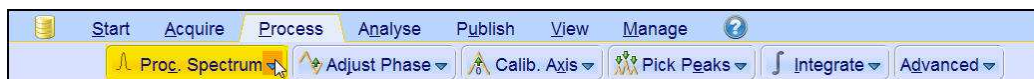

- Select the **Go** button in the Workflow button bar by clicking on it. As a default from the selected experiment, the spectrometer performs a number of dummy-scans before starting the actual number of scans.

5.10   Processing the Data

A Fourier Transformation is used to convert the FID to a frequency spectrum. The number of points used to form the resulting spectrum is determined by the parameter SI (size). The FID is transformed into a spectrum consisting of SI data points in the real part and SI data points in the imaginary part. The normal setting for SI is SI = TD/2. If you have loaded the parameter set "PROTON" you can easily verify that TD = 64K and Si = 32K.
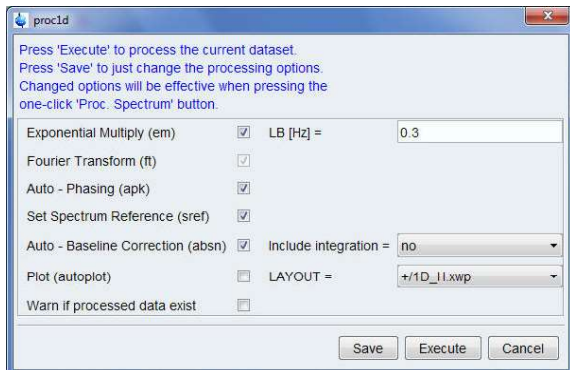
1. Click on the **Process** tab in the Workflow tab bar.

2. Click on the **down arrow** inside the **Proc. Spectrum** button in the Workflow button bar.
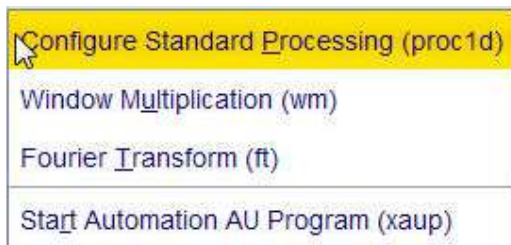
3. Select **Configure Standard Processing (proc1d)** by clicking on it.

4. Enable the following options:
   - **Exponential Multiply (em)**
   - **Auto – Phasing (apk)**
   - **Set Spectrum Reference (sref)**
   - **Auto – Baseline Correction (absn)**

5. Click on **Execute** inside the proc1d window

Note: Use the **Save** button inside the proc1d window to save the processing settings.

5   Automation   w/Auto sampler

5.1   Starting the Automation Setup window

ℹ️ Automation is designed to run experiments using a variety of sample changers and can also be used in manual sample change operation allowing greater flexibility than the Routine Spectroscopy (Chapter. 4).

1. In the TopSpin Menu bar click on the **Acquire** tab.

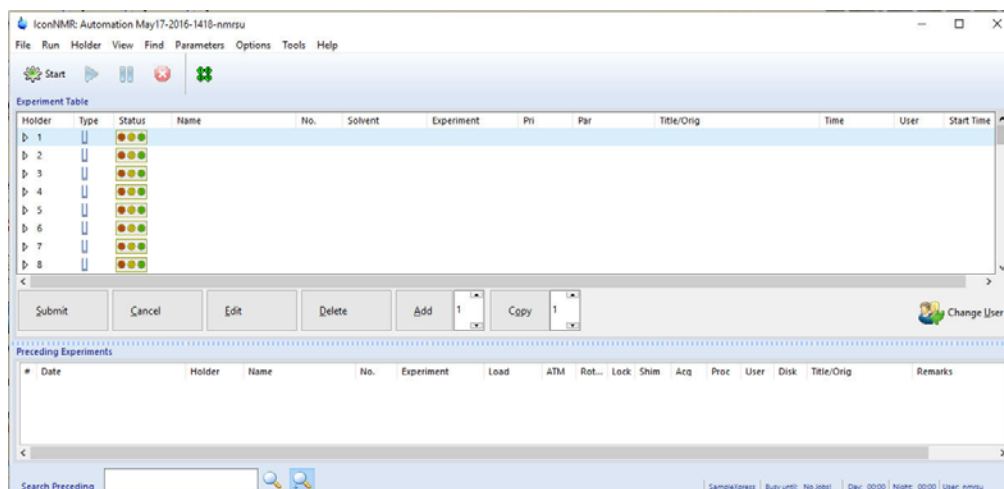2. Click on the down arrow inside the **More** button.

3. Select **IconNMR Automation**

ℹ️ Alternatively, you can also type **icona** on the TopSpin command line.

4. Enter the User password.

5. Hit Enter.

5.1.1 Sample preparation

> The following setup examples are simple exercises to demonstrate the different acquisition methods in IconNMR using 4 samples.

5.1.1.1 Samples

30 mg Cholesterylacetate in 0.6 ml CDCl3

30 mg Carvone in 0.6 ml Acetone-d6

30 mg Strychnine in 0.6 ml CDCl3

**0.1% Ethylbenzene in CDCl3 (Standard Sample)**

5.2 Experiment Setup

5.2.1 Set up of one single experiment

1. Double click on **Holder 1**

2. Click inside the Name window and change the name to **Sample1**

10000055108

> ℹ The experiment number automatically increments to the next available number if a data set already exists with that name and experiment number.

3. Click on the down arrow next to the Solvent window and select **CDCl3**.

4. Click on the down arrow next to the Experiment window and select **PROTON**.
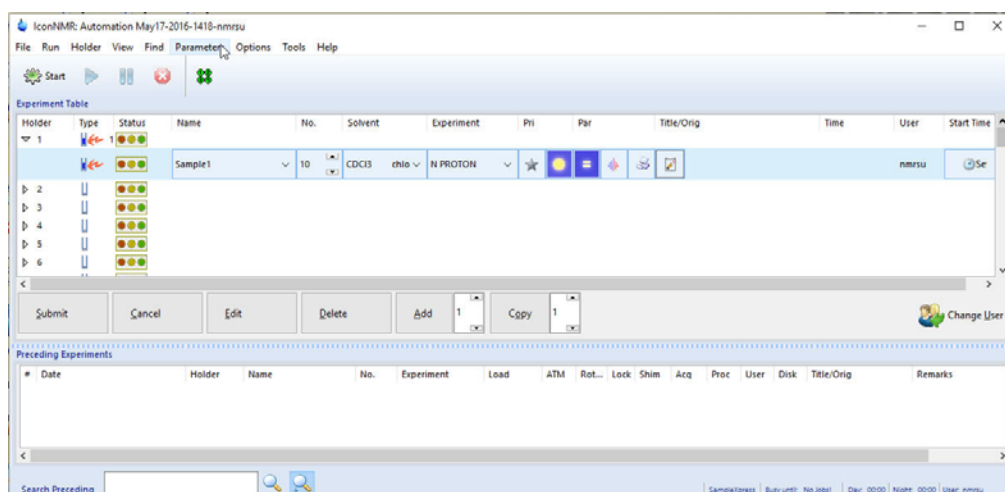
> ℹ Depending on the user's permissions, parameters of the experiment can be changed using the following options.

5.2.1.1   Change all parameters

5. In the IconNMR menu bar, click on **Parameters** and choose any of the options shown in the figure below.
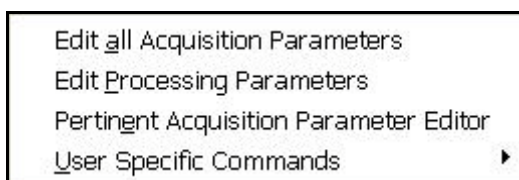
6. Select e.g. **Edit all Acquisition Parameters**.

7. Change any of the parameters in the TopSpin AcquPars window shown in the figure below.
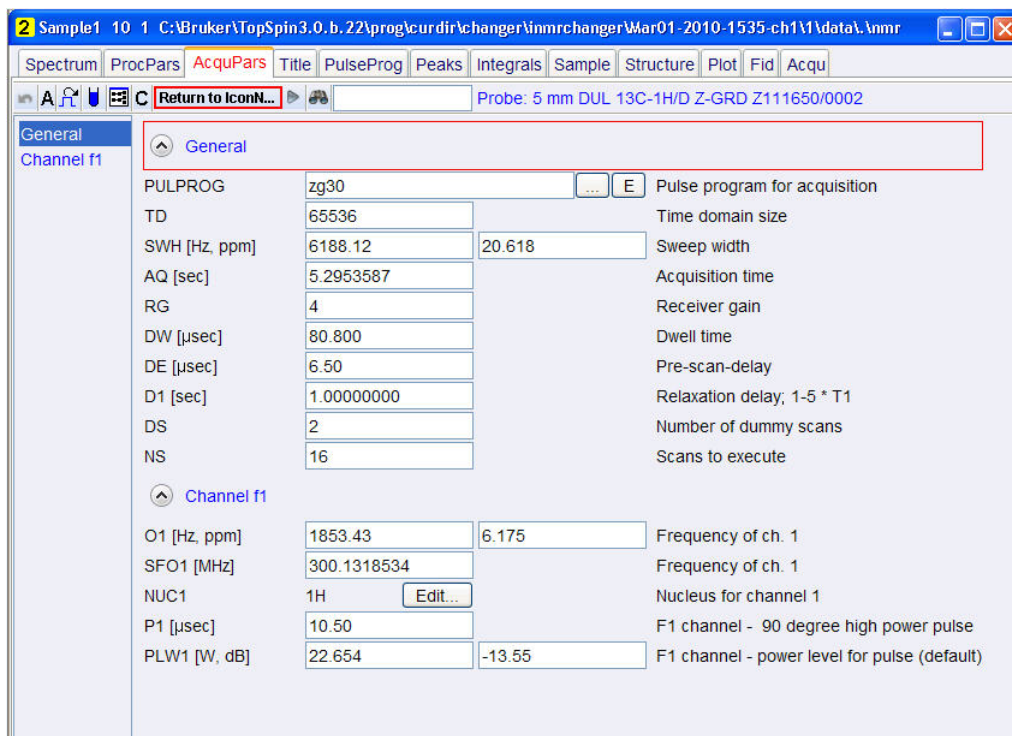
8. Click on the **Return to IconNMR** button to return to the Automation set up window.
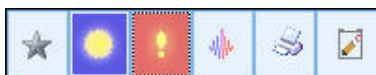
5.2.1.2   Edit User specific parameters

9. Click on the **Edit specific parameters** button.

> Any of the displayed parameters can be changed. If done so, the icon of the Edit specific parameters button will change as illustrated in the picture below.

5.2.1.3   Control Locking Shimming and ATM

10. Click on the Control Locking, Schimming and ATM button to change any options in the figure below.

Lock/Shim Settings

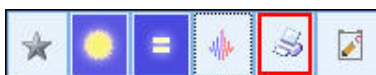| Lock Program | LOCK |
| Shim Program | TOPSHIM ; |

ATM Controls

| ¹H Channel Tuning/Matching | Yes | No | after a solvent change |
| X Channel Tuning/Matching | Yes | No | after a solvent change |

OK

11. Click on the **OK** button inside the Lock.Schim Settings window, when done

5.2.1.4   Toggle Printing

12. Select the **printer** icon for printing the spectrum or select the **green tree** icon for no print.

5.2.1.5   Setting the Title

13. Click on the Set a title button.

14. Type a title e.g. **Cholesterylacetate**

15. Click on the **Set Title** button inside the Title window.

16. Click on the first line of **Holder 1**

17. Click on the Submit button.

> The user may be requested to fill in the originator information before the sample can be submitted. Proceed to paragraph **5.4 Sample run** to start the automation in this chapter.

5.2.2   Set up of two single experiments

1. Follow steps **1** through **17** in paragraph **5.2.1 Setup of one single experiment**.
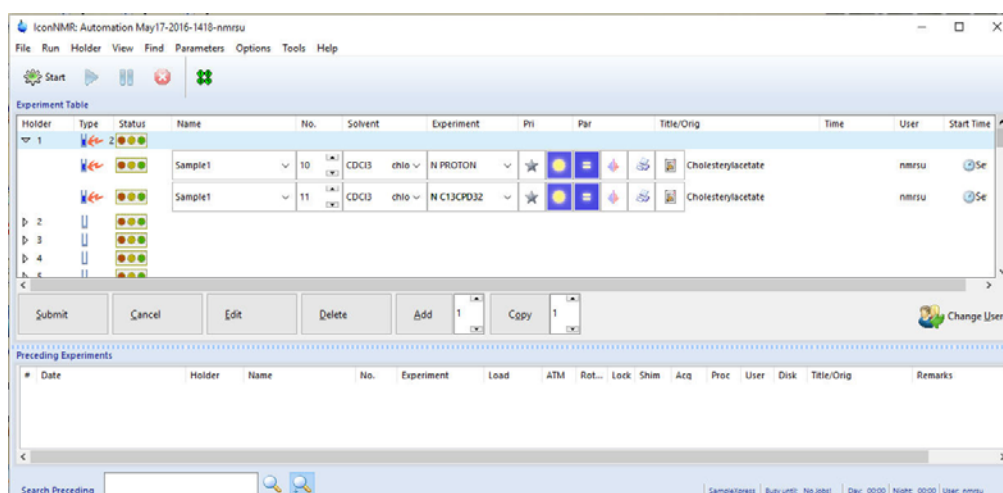

2. Click on the **Add** button.

> ℹ To add one experiment, regardless if it is a single or composite one, a "1" should be displayed in the window next to the Add button. A new line opens up containing the same information for the Disk, Name, Solvent and Orig/Title. The Exp No is incrementing by 1.

3. Click on the small button to the right of the Experiment window and select **C13CPD32**.

4. Click on Holder 1

5. Click on the **Submit** button.

> ℹ The Status traffic lights of the two experiments change to yellow to indicate the holder is ready to run and the experiment time of each experiment is calculated. Proceed to Paragraph 5.4 Sample run to start the automation.
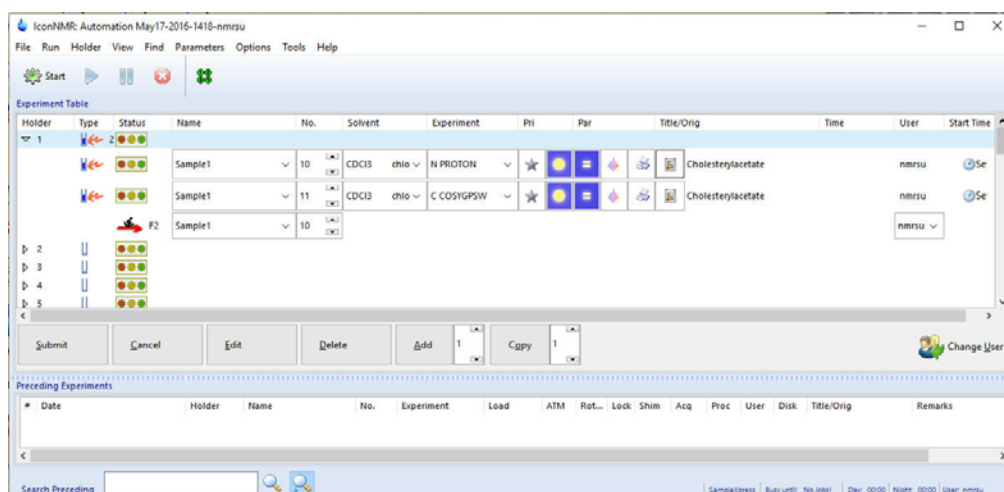
5.2.3 Set up of a composite experiment

1. Double click on **Holder 1**.

2. Click inside the Name window and change the name to **Sample1**.

i

The experiment number automatically increments to the next available number if a data set already exists with that name and experiment number.

3. Click on the down arrow next to the Solvent window and select **CDCl3**.

4. Click on the down arrow next to the Experiment window and select **COSYGPSW**

i

A letter C will be displayed to mark the COSYGPSW to be composite experiment. A composite experiment contains two or more single experiments. In this case, the COSYGPSW is a 2D Homonuclear Correlation experiment which needs a PROTON experiment to optimize the parameters. The PROTON experiment is automatically entered into the present holder.

5. Select and highlight the PROTON experiment.

6. Click on the **Set a title** button.

7. Type a title e.g. **Cholesterylacetate**

8. Click on the **Set & Copy Title** button inside the Title window.

9. Click on the first line of Holder 1.


10. Click on the **Submit** button.

The user may be requested to select a Disk Unit or to fill in the originator information before the sample can be submitted.

The Status traffic lights of the two experiments are changing to yellow to indicate the experiments are in the queue and the experiment time of each experiment is calculated.

Proceed to 5.4 Sample run to start the automation.

5.2.4   Set up of two composite experiments

1. Follow steps 1 through 9 in 5.2.3 Setup of a composite experiment.
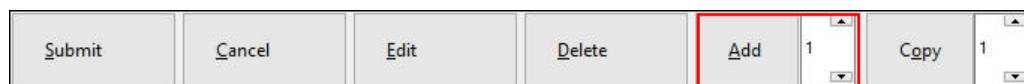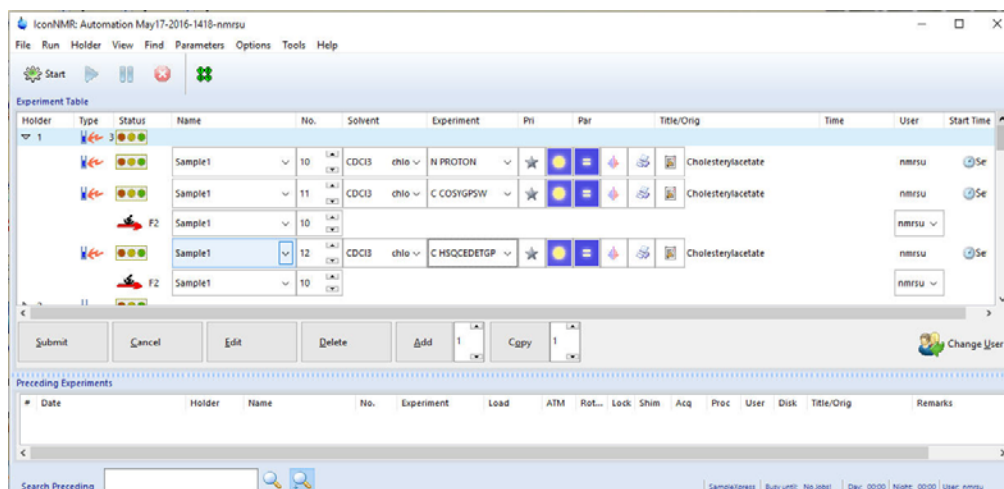

2. Click on the **Add** button.

To add one experiment, regardless if it is a single or composite experiment, a "1" should be displayed in the window next to the Add button. A new line opens up which contains the same information for the Disk, Name, Solvent and Orig/Title. The Exp No is incrementing by 1.

3. Click on the small button to the right of the Experiment window and select **HSQCEDETGP**.

HSQCEDETGP is a composite experiment like COSYGPSW and is marked with the letter C. It is a 2D Hetero inverse correlation experiment which needs a Proton 1D-preparation experiments to optimize the 2D parameters in F2. Since the PROTON experiment already exists, it will use this experiment to optimize the 2D parameters.
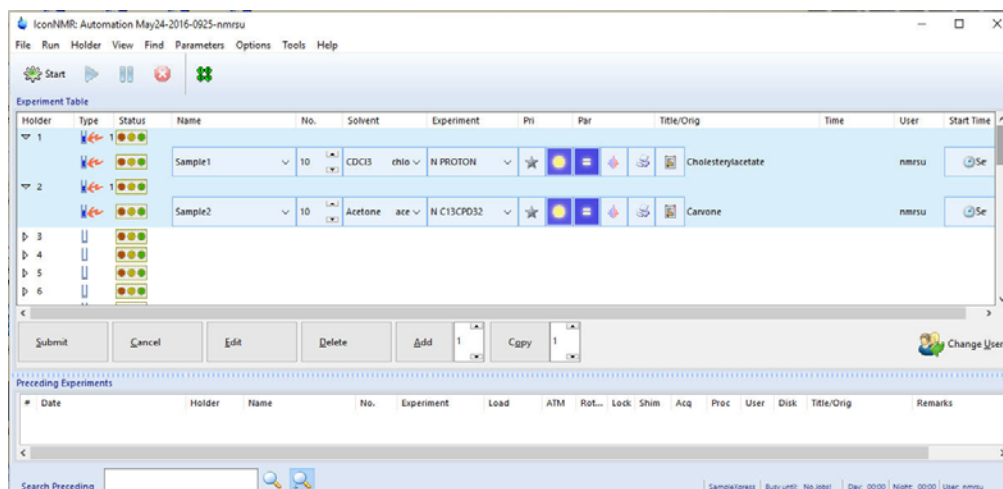
4. Click on the first line of Holder 1.

5. Click on the **Submit** button.

ℹ️ The Status traffic lights of the four experiments change to yellow to indicate the holder is ready to run and the experiment time of each experiment is calculated. To start the Automation Run, proceed to Paragraph 5.4 Sample run to start the Automation.

5.2.5 Set up of two or more holders with different solvents and experiments

1. Follow steps 1 through 17 in 5.2.1 Setup of one single experiment.

2. Double click on Holder 2.

3. Click inside the Name window and change the name to **Sample2**

4. Click on the down arrow to the right of the solvent window and select **Acetone**.

5. Click on the down arrow to the right of the Experiment window and select **C13CPD32**.

6. Click on the **Set a title** button.

7. Type a title e.g. **Carvone**.

8. Click on the **Set Title** button inside the Title window.

9. Click on the first line of Holder 1.

10. Hold down the **Shift** key and click on the last experiment line of Holder 2.

11. Click on the **Submit** button.

> ℹ️ The Status traffic lights change to yellow to indicate the holders are ready to run and the experiment time is calculated.   Proceed to Paragraph 5.4 Sample run to start the automation.
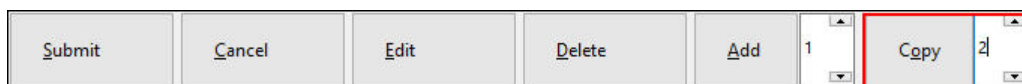
5.2.6 Set up of two or more holders with the same solvents and experiments

1. In the menu bar click on **Holder** and select **Increment Name.**
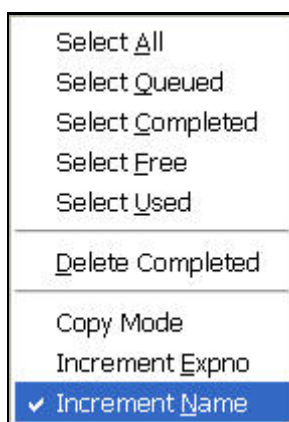
2. Follow steps **1** through **17** of **5.2.1 Setup of one single experiment**.

3. Click on the first line of Holder 1.

4. Increment the number next to the **Copy** button to **2**.

5. Click on the **Copy** button.

To copy the setup from one holder into another holder click on the **Copy** button. The number displayed in the window next to the Copy button is to how many holders it will copy. For this example use **2**. The next two holders will have the same setup as the previous one except that the Name **Sample1** increments to **Sample2** and **Sample3**.

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6. Click on the experiment line in Holder 2.
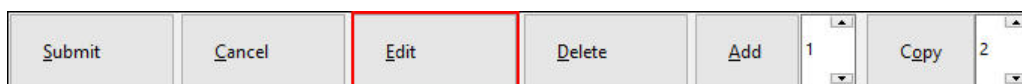
7. Click on the **Edit** button.

8 Click on the **Set a title** button.

9. Type a title e.g. **Ethylbenzene**.

10. Click on the **Set Title** button inside the Title window.

In addition to the title all other entries can be edited in the new holder by clicking on **Edit**. DO NOT CHANGE the Exp No, unless you are sure the new Exp No does not already exist.

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11. Click on the experiment line in Holder 3.

12. Click on the **Edit** button.

13. Click on the **Set a title** button.

14. Type a title e.g. **Strychnine**.

15. Click on the **Set Title** button inside the Title window.

16. Click on the first line of Holder 1.

17. Hold down the **Shift** key and click on the last experiment line of Holder 3.
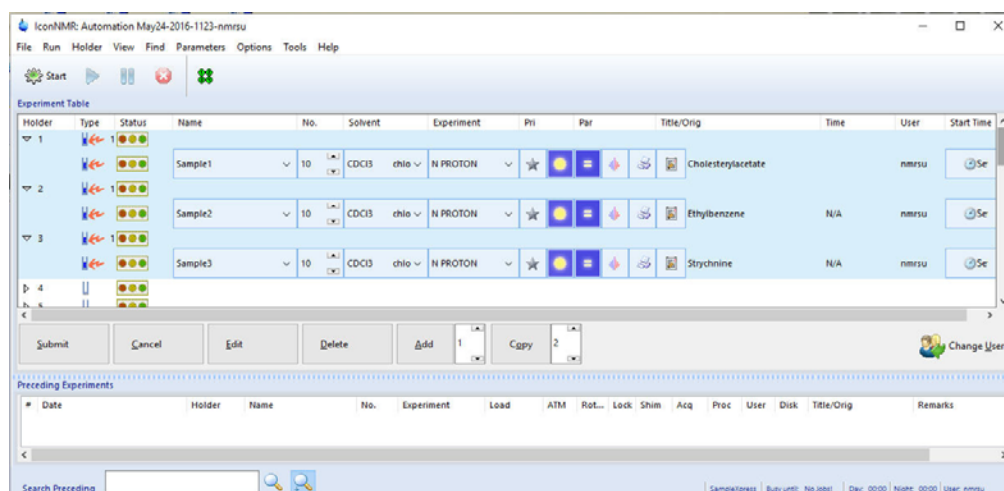
18. Click on the **Submit** button.

> ℹ The Status traffic lights on both holders change to yellow to indicate the holder's ready to run and the experiment time for each experiment is calculated. Proceed to paragraph 5.4 Sample run to start the automation.