OPERATION OF THE INOVA 400 AND 600

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BASIC OPERATION OF THE INOVA 400 AND 600

1. INTRODUCTION

1.1. About these Notes and Related Notes

These notes describe the use of the Inova 400 and Inova 600 spectrometers to perform basic 1D and 2D NMR experiments. Several other sets of notes are available and should be consulted in addition to these notes.

Table 1. Required and Recommended Notes for Performing NMR Experiments on the Inova 400 and 600

If you would like to…	Then you …	
	…must consult these notes	…should consult these notes
Perform a 1D ^{1}H, ^{13}C ^{19}F, or ^{31}P experiment	• Reserving NMR Time using FACES • NMR Sample Preparation • NMR Spectrometer Capabilities and Specifications • Retrieving your NMR Data	• Processing, Analyzing, and Plotting 1D NMR Spectra using VNMRJ
Perform a 1D ^{11}B, ^{77}Se or other odd nuclei experiment	• Reserving NMR Time using FACES • NMR Sample Preparation • NMR Spectrometer Capabilities and Specifications • Performing Odd-Nuclei NMR Experiments on the Inova 400 and 600 • Retrieving your NMR Data	• Processing, Analyzing, and Plotting 1D NMR Spectra using VNMRJ
Perform variable–temperature 1D experiments	• Reserving NMR Time using FACES • NMR Sample Preparation • NMR Spectrometer Capabilities and Specifications • Performing Variable-Temperature NMR Experiments on the Inova 400 and 600 • Retrieving your NMR Data	• Processing, Analyzing, and Plotting 1D NMR Spectra using VNMRJ
Perform a 2D experiment	• Reserving NMR Time using FACES • NMR Sample Preparation • NMR Spectrometer Capabilities and Specifications • Retrieving your NMR Data	• Processing, Analyzing, and Plotting 2D NMR Spectra using VNMRJ

1.2. Capabilities of the Inova 400 and Inova 600

A brief summary of the capabilities of the Inova 400 and Inova 600 in comparison to the Mercury 400 are presented here. For a full discussion, please see the "NMR Spectrometer Capabilities and Specifications" sheet.

Table 2. Capabilities of the Mercury 400, Inova 400 and Inova 600

Spectrometer	^{1}H	^{13}C	^{19}F	^{31}P	Other nuclei	Variable Temperature
Inova 400	Yes	Yes★	Yes	Yes★	Yes	–80 °C to +130 °C^a
Inova 600	Yes★	Yes	No	Yes^b	Yes^b	–20 °C to +80 °C
Mercury 400	Yes	Yes	Yes	Yes	No	No

★Preferred spectrometer
^{a}Requires the use of liquid nitrogen for cooling.
^{b}Requires re-cabling of the probe.

1.3. Specific Inova Policies

1) For standard experiments, no more than two hours of consecutive time can be booked on weekdays between 9:00 am – 6:00 pm. Upon approval from an NMR facility staff member, a maximum of 3 hours is permitted for variable-

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temperature experiments and for samples requiring numerous experiments in which the user must remain at the spectrometer at all times (eg. for titrations, kinetics studies, etc.).

2) From Sunday through Thursday, overnight experiments must start after 6:00 pm and finish by 9:00 am the following day.
3) Odd nuclei or variable-temperature experiments are not to be performed until training has been received from NMR facility staff.
4) Under no circumstances are users allowed to book time using another user's FACES account.
5) **On the Inova 600 only.** Between 11:00 am and 1:00 pm on weekdays, users are limited to a maximum of 30 minutes.

2. GETTING STARTED

2.1. Training

In order to use the Inova 400 or 600, all users must receive training from an NMR facility staff member. Training sessions are regularly offered in January, May, and September, and also upon request by contacting an NMR facility staff member in person or via e-mail at chemnmr@uwo.ca. Upon completion of training, users will be given a FACES account, which can be used to book time on the Inovas.

2.2. Sample Preparation

Follow the directions outlined in the "NMR Sample Preparation" worksheet.

2.3. Reserving NMR Time

1) NMR time can be reserved using the FACES website, http://faces.ccrc.uga.edu. Please consult "Reserving NMR Time using FACES" for more information.

3. LOGGING INTO VNMRJ AND AN OVERVIEW OF THE SOFTWARE

3.1. Logging into VNMRJ

1) Please arrive promptly at the spectrometer.
2) At the VNMRJ welcome screen on the spectrometer's computer, select your group's name from the drop-down list.
3) Enter your group's password and then hit the enter key.

3.2. Overview of the VNMRJ Software

This section provides an overview of the VNMRJ software, highlighting the most important buttons and features.

a) Experiment Panel: *Panel containing all possible NMR experiments. Used to select your experiment.*
b) Study Queue Window: *Contains button that initiate the study queue. The study queue allows the user to perform multiple experiments on the same sample in an automated fashion.*
c) Status Indicators: *Buttons that display the temperature (in °C), the spin-rate (in Hz), and lock level. The values will be blue when regulated, purple when trying to regulate, and orange when not-regulated (or off).*
d) Spectrometer Status Window: *Indicates whether the spectrometer is idle or performing a task. Turns blue when an experiment is running and indicates the time remaining in the experiment.*
e) Information Panel Pop-Up Button: *Button that opens a pop-up window that lists all the messages displayed in the Information Panel.*
f) Information Panel: *Displays a variety of useful messages and also error messages (if any).*
g) "Action" Buttons: *A few of the most commonly used buttons. The buttons change depending on whether the Start, Acquire, or Process panel is selected.*
h) Panels: *Panels containing pages that are used to operate the spectrometer. There are three main panels, Start (which contains to set the conditions before the experiment can begin), Acquire (which contains pages that set all the acquisition parameters and to save your data) and Process (which contains pages that set all the processing parameters and plots your spectrum).*

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i)   Graphics Display: *Window displaying your spectrum or FID.*
j)   Graphics Controls: *A variety of buttons that controls what is displayed in the graphics display and the appearance of your spectrum or FID in the graphics display. Allows you to zoom-in and out, perform integration, etc. See the "Processing 1D NMR Spectra using VNMRJ" and "Processing 2D NMR Spectra using VNMRJ" worksheets for more information.*
k)   Command Line: *Area in which text commands may be entered in order to control the spectrometer, perform experiments, process data, etc.*
l)   Current Experiment: *Displays the current experiment.*
m) Workspace/Experiment Number: *Displays the current experiment number/workspace. Multiple workspaces may be utilized at the same time. See the "Processing 1D NMR Spectra using VNMRJ" worksheet for more information.*
n)   Experiments Menu: *A menu that experiments may be selected from. In some instances, it is preferable to use the Experiment Menu instead of the Experiment Panel.*
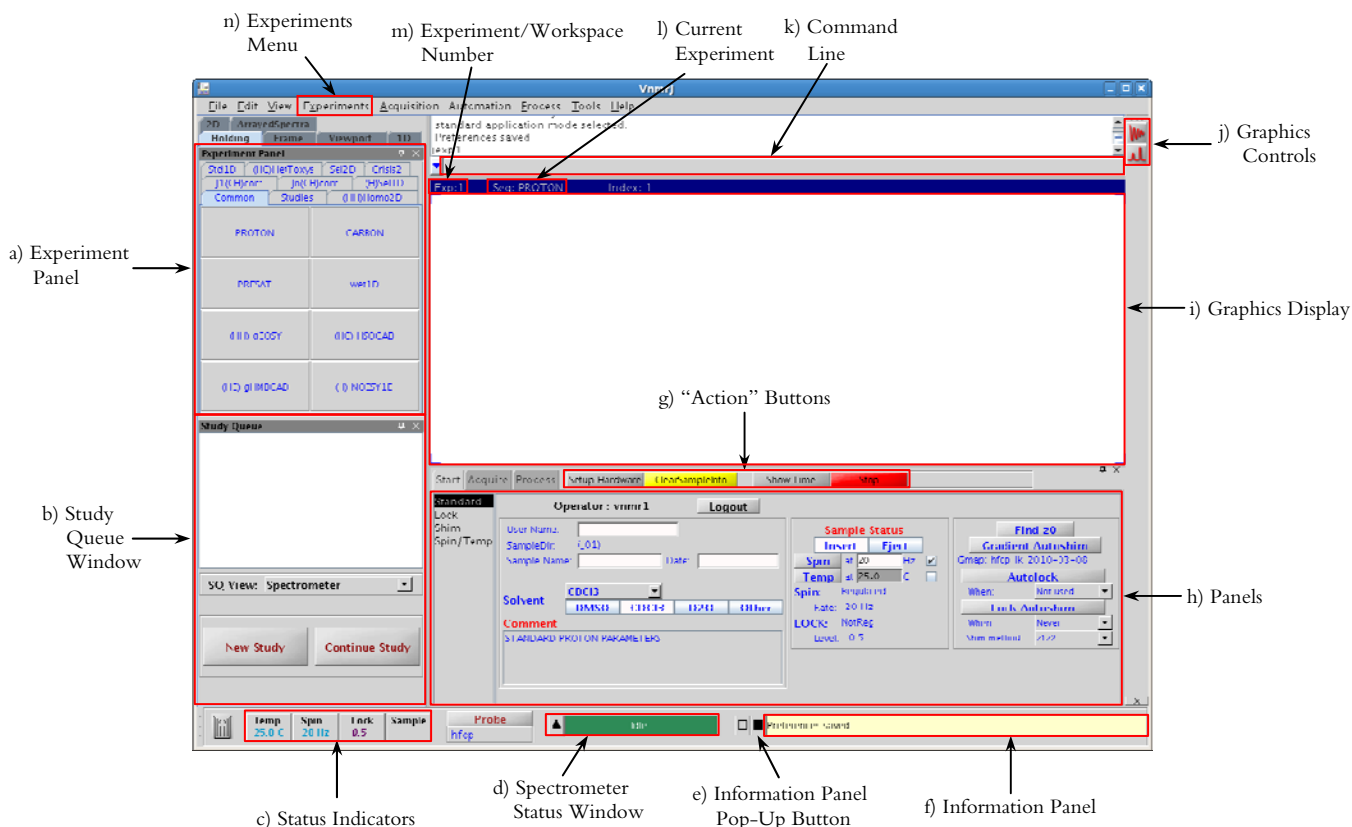

4. INSERTING, LOCKING, AND SHIMMING YOUR SAMPLE

4.1. Inserting your Sample and Selecting your Solvent

1)   Place the empty spinner into the brass depth gauge and push your NMR tube through the spinner until it reaches the bottom of the depth gauge.
2)   Remove the NMR tube and spinner and clean the bottom of the NMR tube with a Kimwipe.
3)   Re-check the depth of the NMR tube in the spinner using the depth gauge.
4)   Click on the **Start** panel and then select the **Standard** page.

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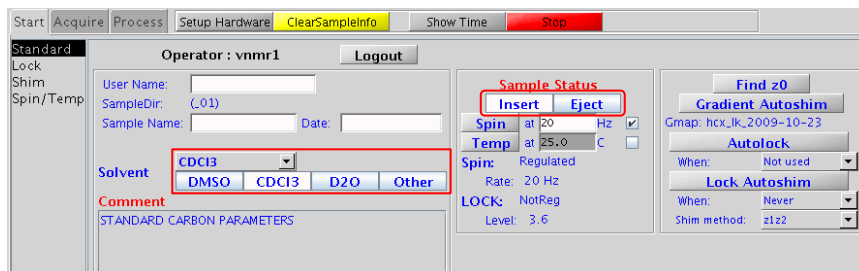
5) Press [Eject]. You should hear air flowing from the spectrometer and the standard CDCl₃ sample should eject.

> **CAUTION:** The eject air MUST ALWAYS be initiated in order to insert a sample, regardless of whether a sample is in the magnet or not.

6) Walk up to the spectrometer (and up the ladder if necessary), remove the CDCl₃ sample and insert your sample in its place.
7) Place the CDCl₃ sample into the metal NMR sample holder.
8) Back at the computer, press [Insert]. The eject air will slowly dissipate and your sample will smoothly drop into place.
9) Select your solvent from the solvent drop-down list.

4.2. Locking and Shimming your Sample

1) Select the **Lock** page.
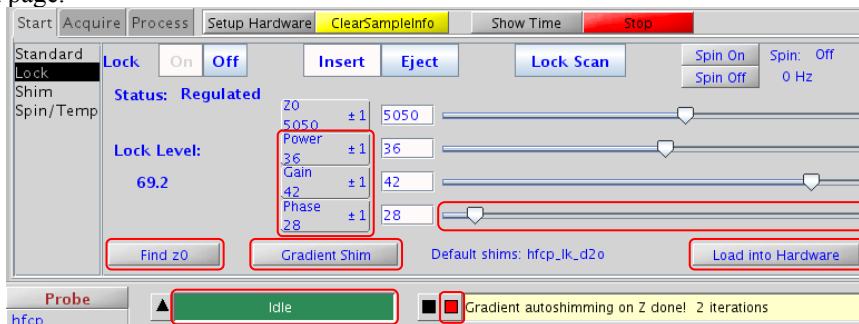
2) Click [Load into Hardware], which loads in the default shim file. The status window will display "Active" and then return to "Idle". Do not proceed to the next step until the "Idle" message is displayed.
3) Click [Find z0] to find the ^{2}H lock frequency and get your solvent on resonance. The status window will turn blue for a few seconds and then return to green and display "Idle". Do not proceed to the next step until the "Idle" message is displayed.
4) Click [Gradient Shim]. The status window will turn blue for a few minutes and then return to green and display "Idle". Do not proceed to the next step until the "Idle" message is displayed.
5) Check that your lock level is between 30 and 70 units.
   a) If so, then proceed to the next section, "5. Performing Basic 1D Experiments Manually".
   b) If the lock level is only slightly below 30, alternately increase the lock power and lock gain by right-clicking on the power and gain buttons, respectively. The middle mouse button will change the increment (ie. From ±1 to ±10).
   c) If the lock level is above 70, alternately decrease the lock power and lock gain by left-clicking on the power and gain buttons, respectively.
   d) If the lock level is near 0, verify that:
      i) You are using a deuterated solvent.
      ii) You have selected the correct solvent. Go to the **Standard** page and check.
      iii) You have done all the correct steps and the gradient shimming has converged by pressing on ■, which brings up a pop-up information window:

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(1) Look for the line, "shims read from /vnmr/shims/startup", which indicates that you have read in the shims. If this line is not present, start back at step 2 of this section.

(2) Look for the line, "z0 set to #", which indicates that you have pressed the **Find z0** button. If this line is not present, start back at step 3 of this section.

(3) Look for the line ending with, "…shims converged…", which indicates that the gradient autoshimming worked correctly. If this line is not present, start back at step 4 of this section.

iv) The lock phase is set correctly by slowly changing the lock phase using the lock phase button or the lock phase slider until a maximum lock level is obtained.

v) You have a sufficient amount of solvent; the tube should be filled to 50 mm from the bottom.

vi) Your sample is not broken. Eject the sample and check.

CAUTION: Do not crank up the lock power and gain to the maximum values. A very low lock level is a symptom of another problem.

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e) If you still do not have an appropriate lock level or have trouble manually locking, please see an NMR facility staff member.

4.3. The Next Step

NMR experiments can be performed manually, in which each experiment is started by the user or in a sample queue, where the experiments are queued and are run sequentially without any further user input. A sample queue is very useful when multiple experiments are to be performed on the same sample. Note that both 1D and 2D NMR experiments can be performed in a sample queue. Instructions on how to perform a 1D experiment manually are presented in Section 5, to perform a 2D experiment manually see Section 6 and on how to perform a sample queue see Section 7.

5. PERFORMING BASIC 1D EXPERIMENTS MANUALLY

5.1. Selecting your Experiment

1) Select the desired experiment from the **Experiments** menu in the top toolbar or from the **Experiment Panel** located on the left-hand side of the screen. If you use the **Experiment Panel**, PROTON and CARBON experiments are located under the **Common** and **Std 1D** tab, while the FLUORINE and PHOSPHORUS are located only under the Std 1D tab.

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Experiments Menu   Experiment Panel: Common Tab   Experiment Panel: Std1D Tab

5.2. Fill out the Sample Information

1) Go to the **Start** panel, **Standard** page.

2) Fill out the information needed to save your data. Your data will be saved in the folder "/home/data/group name/UserName/SampleName_Date"
   a) Complete the "User Name:" section.
   b) Complete the "Sample Name:" section.
   c) Complete the "Date:" section.

3) *(Optional)* Enter any information in the "Comment" section. This section is free-form and can contain any information you want.

5.3. Modifying the Acquisition Parameters

1) Go to the **Acquire** panel, **Default** page.

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2) The acquisition parameters that can be modified depend on the chosen experiment, but generally at least two of the following choices are available:
   a) Spectral width: Determines the chemical shift range of the spectrum.
   b) Number of scans: Determines the number of times the experiment is repeated. The more scans, the longer the experiment takes, but the better the signal-to-noise of your spectrum.
3) *(Optional)* Click ⬜ Show Time to see how long your experiment will take. The time will be displayed in the Information Panel.

⬛⬛ Total acquisition time is 23 sec
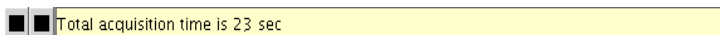
4) Click ⬜ Acquire to start your experiment.
5) Your spectrum will automatically save and be displayed when the experiment is finished. The message, "FID saved: …" should appear in the Information Panel.

⬛🟥 FID saved: /home/data/vnmr1/Mat/3heptanone_20Nov0901/PROTON01
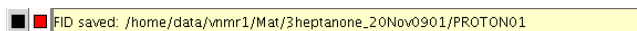
## 5.4. Monitoring and Stopping your Experiment *(Optional)*

1) As the experiment proceeds, the time remaining and the number of scans/transients completed (CT) are displayed in the Spectrometer Status Window at the bottom of the screen.

🔼 **FID: 1 CT: 2 00:00:15**

2) If you would like to see your spectrum before the experiment is completed, go to the **Process** panel. It doesn't matter which page is selected.
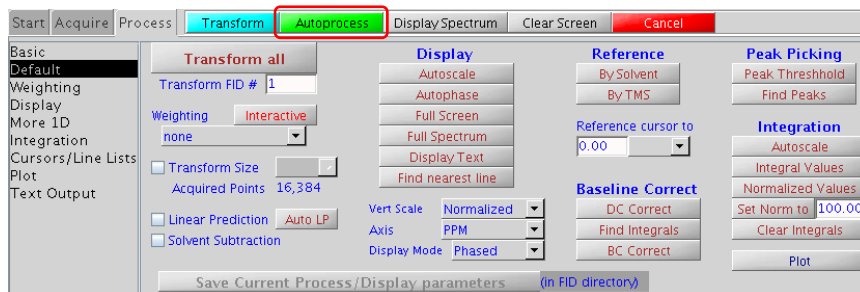
3) When you see the message, "exp#: BS # completed" (it doesn't matter which number it is) in the Information Panel, click on ⬜ Autoprocess. Your spectrum will be displayed in the Graphics Display window.

🟥🟥 exp1: BS 1 completed
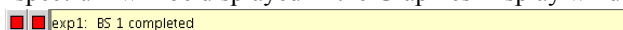
4) If you are happy with your spectrum and do not wish to complete all the scans, go to the **Acquire** panel and click on ⬜ Stop.
5) Your spectrum will NOT automatically save when the experiment is finished. In order to save your data, follow steps 4 – 6 of section 5.5.

5.5. Saving your Data Manually *(Optional)*

If your spectrum has saved in the incorrect location, the data can be saved manually using the following procedure.
1) Go to the **Start** panel, **Standard** page
2) Click on ⬜ ClearSampleInfo.
3) Input new information in the User Name, Sample Name, and Date fields
4) Go to the **Acquire** panel, **Future Actions** page.

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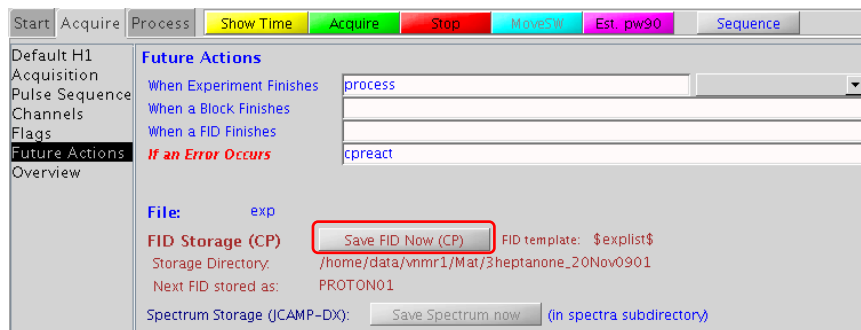
5) The directory in which your data will be saved is displayed next to "Storage Directory". The experimental data file will be saved in the "Storage Directory" and will have name of the experiment appended with a number plus the extension .fid (eg. PROTON01.fid).

6) Click on ⌈ Save FID Now (CP) ⌋. The message, "FID saved: …" should appear in the Information Panel.

5.6. Processing, Analyzing, and Plotting your Spectrum

Please see the "Processing 1D Spectra using VNMRJ" worksheet.

5.7. Performing Additional Experiments

1) If you have additional 1D experiments to perform on the *same* sample, go back to the start of this section (section 5) for each additional experiment you wish to perform.

2) If you have 2D experiments you would like to perform on the *same* sample, move onto the next section (section 6).

3) If you have additional experiments to perform on a *different* sample, begin at section 4.

4) If you have no more experiments you wish to perform, move onto section 8.

6. PERFORMING BASIC 2D EXPERIMENTS MANUALLY

The previous section discussed performing 1D experiments manually. This section deals with performing 2D experiments manually. Performing 2D experiments is the same as performing 1D experiments, except for one additional step. Instructions on how to perform 2D experiments as part of a sample queue can be found in the next section (section 7).

6.1. Narrowing the ^{1}H Sweep–Width

1) If you have already completed a PROTON experiment on your sample, go to **File → Open**, navigate to your spectrum and double-click on the PROTON0#.fid file.

2) If you have not performed a PROTON experiment, follow the steps described in section 5, selecting PROTON as your experiment.

3) After your experiment has completed, use the following procedure to zoom-in on just the peaks of interest:

   a) Click on ⌈⌉ on the top-right of the screen to display the full spectrum.

   b) Left-click just to the left of the most downfield peak in your ^{1}H spectrum.

   c) Right-click just to the right of the most upfield peak in your ^{1}H spectrum.

   d) Click on ⌈ MoveSW ⌋, which narrows the sweep-width about your peaks of interest. If you receive an error message, type **crmode='b'** in the command line, then click on ⌈ MoveSW ⌋.

6.2. Selecting and Starting your Experiment

1) Select the desired experiment from the **Experiments → Convert current parameters to do…** menu in the top toolbar. **DO NOT** use the **Experiment Panel** located on the left-hand side of the screen.

2) Follow the steps listed in sections 5.3, 5.4, 5.5, 5.6, and 5.7, except for section 5.6 see "Processing, Analyzing, and Plotting your 2D Spectrum using VNMRJ" instead.

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> **CAUTION:** 2D experiments must be run on a non-spinning sample. The spinning rate is displayed in the **Spin Status Indicator** on the bottom left-hand side of the screen. After you hit the **Acquire** button the spin-rate should read zero. If your sample is spinning, click on the **Stop** button, go to the **Start** panel, **Spin/Temp** page and click on the **Spin Off** button. Go back to the **Acquire** panel and click on the **Acquire** button to re-start the experiment.

## 7. PERFORMING EXPERIMENTS USING THE STUDY QUEUE

7.1. Preparing your Sample

1) Click on [New Study] on the left-hand panel of VNMRJ.
2) Go to the **Start** panel, **Standard** page.

3) Fill out the information needed to save your data. Your data will be saved in the folder "/home/data/group name/UserName/SampleName_Date"
   a) Complete the "User Name:" section.
   b) Complete the "Sample Name:" section.
   c) Complete the "Date:" section.
4) *(Optional)* Enter any information in the "Comment" section. This section is free-form and can contain any information you want.
5) Choose whether you want the spectra to automatically print after completion by selecting or deselecting the Autoplot box.
6) Ensure that autolock and gradient shimming are not selected. These are located in the middle of the main panel, under the text "Before First EXP".
   a) In the drop-down list next to Lock?, select "No (alock = n)".
   b) Deselect the box labeled, "Shim".

7.2. Selecting and Customizing your Experiments

1) Select your experiments from the Experiment Panel on the top-left of the screen by clicking on the experiments in the order that you wish them to be performed. The experiment, time required for each experiment, and the total time for the sample queue will be displayed on the left-hand side.

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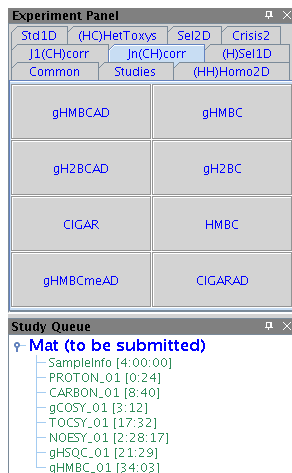
2) **(Optional)** Customize your experimental parameters.
    a) Double-click on the experiment in the list of chosen experiments (not the Experiment Panel). This reads in the experiment.
    b) Go to the **Acquire** panel, **Default** page and modify any parameters that you wish.
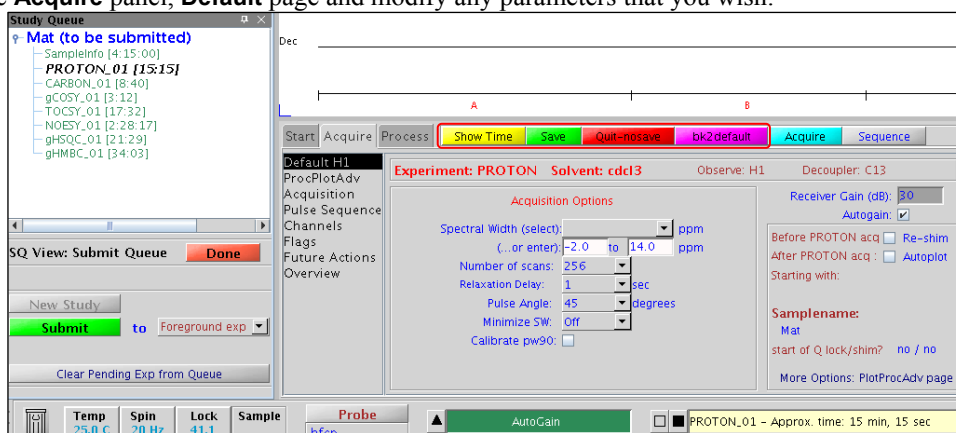
    c) If desired, click **Show Time**. The experiment time will be updated and displayed in the experiment list.
    d) Then choose one of the following options:
        i) **Save** to save the modified parameters and return to the previous screen.
        ii) **Quit-nosave** to cancel the modified parameters and return to the previous screen.
        iii) **bk2default** to reset the modified parameters back to the default parameters. You must then hit "Save" or "Quit-nosave" to return to the previous screen.
    e) Repeat steps a – d for any additional experiments that you would like to customize.
3) **(Optional)** Delete unwanted experiments by selecting the experiment from the Experiment List and dragging it into

    on the bottom left corner of the screen. The total sample time will be updated.
4) Start the sample queue by clicking on **Submit**. The experiments will automatically save when completed.
5) If you decide that you do not want to perform the sample queue, click on **Done**.

8. FINAL STEPS

1) Eject your sample and insert the standard $CDCl_3$ sample:
    a) Click on the **Start** panel, then select the **Standard** page.
    b) Press **Eject**. You should hear air flowing from the spectrometer and your sample should eject.
    c) Walk up to the spectrometer (and up the ladder if necessary), remove your sample and insert the standard $CDCl_3$ sample in its place.

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    d)   Press ⏎ Insert . The eject air will slowly dissipate and your sample will smoothly drop into place.

2)   Go to the **Start** panel, **Standard** page and click on the "Logout" button.